

Epidemiology and pathogenesis of Kaposi's sarcoma-associated herpesvirus

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Kaposi's sarcoma (KS) occurs in Europe and the Mediterranean countries (classic KS) and Africa (endemic KS), immunosuppressed patients (iatrogenic or post-transplant KS) and those with acquired immune deficiency syndrome (AIDS), especially among those who acquired human immunodeficiency virus sexually (AIDS-KS). KS-associated herpesvirus (KSHV or HHV-8) is unusual among herpesviruses in having a restricted geographical distribution. Like KS, which it induces in immunosuppressed or elderly people, the virus is prevalent in Africa, in Mediterranean countries, among Jews and Arabs and certain Amerindians. Distinct KSHV genotypes occur in different parts of the world, but have not been identified as having a differential pathogenesis. KSHV is aetiologically linked to three distinct neoplasms: (i) KS, (ii) primary effusion lymphoma, and (iii) plasmablastic multicentric Castleman's disease. The histogenesis, clonality and pathology of the tumours are described, together with the epidemiology and possible modes of transmission of the virus.

Keywords: Kaposi's sarcoma-associated herpesvirus; human herpesvirus 8; Kaposi's sarcoma; body cavity-based lymphoma; primary effusion lymphoma; Castleman's disease

1. EPIDEMIOLOGY OF KAPOSI'S SARCOMA

Nearly 40 million people worldwide are now infected by the human immunodeficiency virus (HIV-1), not counting the 20 million people who have already died from HIV-related diseases. In Africa, where most of these people live, the vast majority of HIV-infected individuals still succumb to infectious diseases, most notably tuberculosis. However, opportunistic neoplasms are also taking their toll and Kaposi's sarcoma, for example, is now the most common tumour in men in Uganda (Ziegler *et al.* 1997).

In 1872, the Hungarian dermatologist Moritz Kaposi published the case histories of five patients with 'idiopathic multiple pigmented sarcomas' of the skin (Kaposi 1872). This form of the disease was eponymously designated Kaposi's sarcoma (KS) in 1891. Kaposi's original patients had a more aggressive disease than HIV-negative cases in Europe today, with tumours described in the pharynx, stomach, small intestine, liver and colon in addition to the skin (Kaposi 1872). It is not known whether these patients were significantly immunosuppressed due to a concurrent illness. Four epidemiological forms of KS are currently distinguished: classic KS in Europe and the Mediterranean; endemic, HIV-negative KS in Africa; post-transplant or iatrogenic KS; and HIV-associated KS.

Classic KS occurs predominantly in elderly patients of southern European, Arabic or Jewish ancestry (Franceschi & Geddes 1995), where it usually remains as an indolent

disease affecting the extremities. It is more common in men than women (sex ratios are estimated to be from 3:1 to 10:1) (Franceschi & Geddes 1995). Within countries where classic KS occurs there is also a specific geographical distribution; e.g. in Greece KS occurs more commonly in those from the Peloponnese peninsula. In Italy, higher incidence rates are reported in Sicily and southern Italy than in northern Italy (Bertaccini 1959; De Amicis 1897). Between 1977 and 1991 the annual incidence of classic KS in Sardinia per 100 000 inhabitants was 2.43 for men and 0.77 for women (ratio 3.2:1) (Cottoni *et al.* 1996). In eastern European countries KS predominates in those of Ashkenazi Jewish heritage (Rothman 1962).

In some equatorial countries of Africa, KS has existed for many decades, pre-dating the emergence of HIV. This form of the disease is known as endemic KS and is usually more aggressive than the classic form (D'Oliveira & Torres 1972). Lymph nodes are often affected and the disease is seen in both children and adults (Olweny *et al.* 1976; Ziegler & Katongole-Mbidde 1996). Whereas the median age of classic KS is 65 years, the median age of endemic KS is 40 years (Oettle 1962). As reports of African endemic KS accumulated during the 1960s and 1970s it became clear that there is also a specific geographical distribution in Africa: KS is more common in equatorial Africa; e.g. Oettle (1962) reported that KS represented 10% of all malignancies in Uganda with a male to female ratio in adults of 15:1.

During the past three decades, the incidence of KS among renal transplant recipients and other patients

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Table 1. *KS incidence from population-based registries*

country	period	male	female	reference
classic KS ^a				
USA	1970–1979	0.29	0.10	Biggar <i>et al.</i> (1984)
UK	1975–1988	0.01	0.003	Grulich <i>et al.</i> (1992)
Israel	1970–1989	1.93	0.73	Iscovich <i>et al.</i> (1998)
Sardinia	1997–1991	2.43	0.77	Cottoni <i>et al.</i> (1996)
Africa in the AIDS era ^b				
Zimbabwe	1990–1992	23.6	10.1	Bassett <i>et al.</i> (1995)
Uganda	1991–1993	48.2 ^c	23.8	Wabinga <i>et al.</i> (1993)
Rwanda	1991–1994	9.5	2.5	Newton <i>et al.</i> (1996)
Guinea	1992–1995	0.2	0.2	Koulibaly <i>et al.</i> (1997)

^a KS incidence per 100 000 population, age-standardized.

^b Percentage of all recorded cancers that are KS.

^c Although this study suggests that nearly 50% of all male cancers in Uganda are due to KS, there could be a bias towards HIV-infected patients attending central hospitals.

receiving immunosuppressive therapy has increased (post-transplant or iatrogenic KS) (Harwood *et al.* 1979). The incidence of KS among renal transplant recipients is estimated to be approximately 150 times that seen in a healthy Western population (Penn 1983). In some countries where endemic or classic KS occurs (e.g. Saudi Arabia), KS is the most common post-transplant tumour (Qunibi *et al.* 1988). This again indicates that specific genetic or environmental factors are involved in its pathogenesis. In Europe, a retrospective study (1968–1990) among 7923 organ-transplant recipients recorded an overall prevalence of KS of 0.52% and KS was significantly more frequent after a liver (1.24%) than a kidney (0.45%) or heart (0.41%) transplant (Farge 1993). Patients of Mediterranean, Jewish or Arabian ancestry are also overrepresented among iatrogenic immunosuppressed patients who develop KS in North America (Franceschi & Serraino 1995). This indicates that those who were born or whose family originated from countries where classic KS occurs continue to be at higher risk of developing KS, even after migration to so called ‘low-risk’ countries.

In 1981 the first cases of KS in young men from New York City and San Francisco were described (US Public Health Service 1981) heralding the beginning of the acquired immune deficiency syndrome (AIDS). KS is now the most common neoplasm occurring in patients with AIDS (Beral *et al.* 1990; Rabkin *et al.* 1995). Like the other forms of KS, AIDS–KS is not distributed randomly among HIV risk groups and surveillance data have consistently supported the existence of a sexually transmissible KS cofactor: in the West, AIDS–KS occurs predominantly in gay men with HIV, less commonly in those who acquired HIV through heterosexual contact and only rarely in patients who acquired HIV parenterally (patients with haemophilia or intravenous drug users) (Beral *et al.* 1990). In Africa, HIV infection has had an immense effect on the incidence of KS and the vast majority of patients with KS live on that continent. Recent reports from sub-Saharan Africa have confirmed the wide differences in incidence rates between countries and also between sexes (table 1).

In Uganda, among HIV-positive subjects, KS cases are characterized by better education and more affluence,

compared with controls (Ziegler *et al.* 1997). Urban, rather than rural address, exposure to water, travel away from home and sexually transmitted diseases are also more frequent among those with KS (Ziegler *et al.* 1997). These data may indicate that poverty protects against KS development. It has been proposed that early childhood infection, with an agent such as KS-associated herpesvirus (KSHV), confers long-lasting immunity, whereas acquisition of such an agent as sexually active adults would be less protective, especially with immunosuppression due to HIV (Ziegler *et al.* 1997).

2. KAPOSI'S SARCOMA HISTOGENESIS

Histologically, KS is a complex lesion: in early lesions there are a collection of irregular endothelial lined spaces that surround normal dermal blood vessels and these are accompanied by a variable inflammatory infiltrate (patch stage). This stage is followed by the expansion of a spindle-celled vascular process throughout the dermis. These spindle cells form slit-like, vascular channels containing erythrocytes (plaque stage). The later nodular stage KS lesions are composed of sheets of spindle cells, some of which are undergoing mitosis, and slit-like vascular spaces with areas of haemosiderin pigmentation. The spindle cells form the bulk of established KS lesions and are therefore thought to be the neoplastic component. Most of the spindle cells in KS lesions express endothelial markers, including CD31 and CD34. However, it was also shown that KS spindle cells express markers for smooth muscle cells, macrophages and dendritic cells (Nickoloff & Griffiths 1989; Sturzl *et al.* 1992) suggesting that spindle cells are either derived from pluripotent mesenchymal precursors or represent a heterogeneous population of cells. Recently, it was shown that all KS spindle cells express vascular endothelial growth factor receptor (VEGFR)-3 (Dupin *et al.* 1999; Jussila *et al.* 1998). VEGFR-3 is usually expressed only by lymphatic endothelium and by neoangiogenic vessels, but not by mature vascular endothelial cells, indicating that KS spindle cells probably belong to the endothelial lineage that can differentiate into lymphatic cells.

Endothelial stem cells have not yet been identified (Risau 1997), but putative endothelial progenitors

(angioblasts) involved in angiogenesis have been isolated from peripheral blood (Asahara *et al.* 1997). These cells express CD34 and the VEGF receptor Flk-1 and might be related to KS spindle cells. Circulating KS-like spindle cells have been isolated and cultured from patients with AIDS-KS and HIV-infected individuals thought to be at risk of developing KS. These circulating cells have an adherent phenotype and express markers for both macrophages and endothelial cells, as well as *in vitro* functional activities similar to KS spindle cells (Browning *et al.* 1994; Sirianni *et al.* 1997).

Tumours produce cytokines and their cells respond positively or negatively to cytokines in culture. KS is no exception and KS spindle cells or infiltrating CD8⁺ lymphocytes and macrophages express high levels of interleukin-6 (IL-6), basic fibroblast growth factor (bFGF), tumour necrosis factor- α , oncostatin M and γ -interferon (IFN- γ) (Ensoli *et al.* 1989, 1994; Fiorelli *et al.* 1998; Miles *et al.* 1990; Nair *et al.* 1992; Salahuddin *et al.* 1988; Samaniego *et al.* 1995). IL-6 is produced by KS spindle cells and exogenous IL-6 enhances the proliferation of KS cells in culture (Miles *et al.* 1990). IFN- γ also induces endothelial cells to acquire similar phenotypic features to KS spindle cells (Fiorelli *et al.* 1998). Because of the nature of KS lesions it has been suggested that these tumours are 'cytokine driven'.

The more aggressive nature of HIV-associated KS has led to speculation that HIV-1-encoded proteins may directly enhance KS growth (Ensoli *et al.* 1994). The HIV-1 Tat protein transactivates HIV viral genes and also some host cell genes (Vaishnaw & Wong-Staal 1991). Tat can be released by infected cells and can act extracellularly (Ensoli *et al.* 1993; Frankel & Pabo 1988). Tat induces a functional programme in endothelial cells related to angiogenesis and inflammation including the migration, proliferation and expression of plasminogen activator inhibitor-1 and E selectin (Albini *et al.* 1995). Tat induces growth of KS spindle cells *in vitro* and is angiogenic *in vivo* and in transgenic mice (Ensoli *et al.* 1993, 1994; Vogel *et al.* 1988). The Tat basic domain contains an arginine- and lysine-rich sequence which is similar to that of other potent angiogenic growth factors including vascular endothelial growth factor-A (VEGF-A) and bFGF (Albini *et al.* 1996). Tat specifically binds to and activates the Flk-1/kinase domain receptor (Flk-1/KDR), a VEGF-A tyrosine kinase receptor (Albini *et al.* 1997). Tat-induced angiogenesis can be inhibited by agents blocking this receptor (Albini *et al.* 1997). The RGD-containing region of Tat has also been postulated to have a role in the pathogenesis of AIDS-KS; however, baboons infected with HIV-2, whose Tat lacks an RGD sequence, can develop 'KS-like lesions', albeit with myofibroblast, rather than endothelial, phenotype (Barnett *et al.* 1994; Ensoli *et al.* 1994). AIDS-associated KS has a specific tissue distribution (often affecting the nose, oral and genital mucosae) and it is possible that the angiogenic or tumour-growth-promoting properties of Tat (Prakash *et al.* 2000) contribute to these phenomena.

The fact that the incidence of KS is higher in African countries where HIV-1 is prevalent, compared with countries where HIV-2 is more common, despite similar prevalences of antibodies to KSHV (Ariyoshi *et al.* 1998), has argued in favour of functional differences between

HIV-1 and HIV-2 Tat proteins (Gallo 1998). Although the increased incidence in HIV-1-infected individuals of various neoplasia including KS, lymphoma and squamous carcinoma is well documented (Parkin *et al.* 1999), we are not aware of definitive cancer statistics from HIV-2-prevalent regions. HIV-1 Tat could indirectly affect tumour cell proliferation via the induction of cellular growth and angiogenic factors and/or directly activate transcriptional regulators like nuclear factor kappa B.

3. KAPOSI'S SARCOMA CLONALITY

The nature of KS also remains controversial as to whether it is a neoplastic lesion or a reactive process. Like Hodgkin's disease, the exact tumour cell type can be argued and the 'tumour cell' compartment in early lesions makes up the minority of the tumour bulk, where the majority of cells are inflammatory cells. Furthermore, the clinical presentation of multiple skin lesions in a defined distribution and the spontaneous remission of lesions favour a reactive hyperplasia rather than a true malignancy.

A useful marker for clonality is the inactivation pattern of X chromosomes in females with cancer (Vogelstein *et al.* 1985). The human X-linked androgen receptor gene can be used to assess clonal patterns of X chromosome inactivation, owing to common polymorphisms which can be identified by methylation-dependent DNA restriction enzymes. Using this technique, Rabkin *et al.* (1995) showed that individual KS lesions are probably clonal. However, a French group led by Oksenhendler demonstrated in skin lesions, including four with nodular KS where more than 70% of the cells were spindle cells, a polyclonal pattern of inactivation (Delabesse *et al.* 1997). Two other studies have indicated that multiple lesions in the same patient can be oligo- or monoclonal (Gill *et al.* 1998; Rabkin *et al.* 1997), suggesting that KS is a disseminated monoclonal cancer and that the changes that permit the clonal outgrowth of spindle cells occur before metastasis. The circulating cells giving rise to multiple clonal lesions are potentially related to the spindle-shaped cells that can be cultured from peripheral blood, which are increased in HIV-infected patients who have KS or are at high risk of developing KS (Browning *et al.* 1994).

Judde *et al.* (2000) expanded a study first explored by the discoverers of KSHV (Russo *et al.* 1996) investigating KSHV clonality within tumours by analysing the size heterogeneity of KSHV-fused terminal repeats (TRs). This assay has been previously used to demonstrate that certain Epstein-Barr virus (EBV)-associated tumours are monoclonal (Raab-Traub & Flynn 1986). However, the TR region of KSHV is much larger, and rearrangements might therefore occur, skewing data towards oligo- or polyclonality. By using this technique Judde *et al.* (2000) showed that KSHV is monoclonal within nodular KS lesions, indicating that the virus was present prior to the expansion of a tumour clone of KS spindle cells.

Early KS (patch stage) is probably a non-clonal proliferation of lymphatic endothelial cells or endothelial precursors (e.g. angioblasts) (Risau 1997) with a prominent inflammatory and angiogenic response, whereas advanced disease can develop into a true clonal malignancy with

metastases of clonally derived spindle cells (Delabesse *et al.* 1997; Gill *et al.* 1998; Rabkin *et al.* 1995, 1997).

4. MOLECULAR DETECTION OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

A viral aetiology for KS was suspected long before the onset of the AIDS epidemic (Oettle 1962). In 1972, herpesvirus-like particles were found by electron microscopy in KS tumour cells and were shown to be cytomegalovirus (CMV) (Giraldo *et al.* 1972, 1975). DNA sequences of CMV, human herpesvirus 6 (HHV-6), human papilloma viruses (HPVs) and BK virus (human polyoma virus) and other viral or bacterial pathogens have all been detected in KS lesions and put forward as suspected aetiological agents. However, these agents, including CMV, HHV-6 and HPV are only found in a minority of the KS lesions (Huang *et al.* 1992; Kempf *et al.* 1995; Monini *et al.* 1996b). Following the discovery of KSHV (or HHV-8) in AIDS-KS biopsies (Chang *et al.* 1994; Moore & Chang, this issue), the vast majority of KS lesions from patients with AIDS and in the other epidemiological groups were shown to be positive for this novel virus (table 2) (Ambroziak *et al.* 1995; Boshoff *et al.* 1995b; Chang *et al.* 1996; Schalling *et al.* 1995). KSHV is now accepted to be the transmissible agent of KS.

KSHV DNA is found in all clinical stages of KS lesions (patch, plaque and nodular), and is generally absent in non-KS tissues from KS patients, in other vascular neoplasms and in other forms of skin tumours from immunosuppressed patients (IARC 1997). In peripheral blood mononuclear cells (PBMC), KSHV DNA can be detected by polymerase chain reaction (PCR) in approximately 50% of KS patients. Further, KSHV genome detection in PBMC of HIV-sero-positive individuals can predict who will subsequently develop KS (Moore *et al.* 1996; Whitby *et al.* 1995). Although one group reported the frequent detection of KSHV in the semen of healthy Italian donors (Monini *et al.* 1996a), KSHV is not detectable in semen donors in North America and the UK and only rarely in patients with KS (Howard *et al.* 1997; Pellett *et al.* 1999; Tasaka *et al.* 1996). Reports of KSHV detection in bone marrow stromal cells of multiple myeloma patients and in sarcoid tissues are also controversial (Di Alberti *et al.* 1997; Rettig *et al.* 1997) and most researchers now believe these are due to PCR contamination (Tarte *et al.* 1998; Whitby *et al.* 1997).

5. KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS MOLECULAR STRAIN VARIATION

Open reading frame (ORF)-K1 is used to subtype KSHV (McGeoch & Davison 1999; Nicholas *et al.* 1998): subtypes A, B, C and D have been identified, and display between 15 and 30% amino-acid differences between their ORF-K1-coding regions. These subtypes have close associations with the geographical and ethnic background of individuals (figure 1). Within these four subtypes, over 15 clades have now been described (Cook *et al.* 1999; Hayward 1999; Lacoste *et al.* 2000a; Meng *et al.* 1999; Poole *et al.* 1999; Zong *et al.* 1999). Subtype B is found almost exclusively in patients from Africa, subtype C in individuals from the Middle East and Mediterranean

Table 2. *Detection of KSHV DNA by PCR in KS biopsies*

(Data compiled from Ambroziak *et al.* 1995; Boshoff *et al.* 1995b; Buonaguro *et al.* 1996; Cathomas *et al.* 1996; Chang *et al.* 1994, 1996; Chuck *et al.* 1996; Dictor *et al.* 1996; Dupin *et al.* 1995; Gaidano *et al.* 1996; Jin *et al.* 1996; Lebbé *et al.* 1995, 1997; Luppi *et al.* 1996; McDonagh *et al.* 1996; Moore & Chang 1995; O'Neil *et al.* 1996; Schalling *et al.* 1995.)

type of lesion	no. positive/ no. tested	per cent positive
AIDS-KS	252/259	97
classic KS	160/175	91
iatrogenic KS	13/13	100
African endemic KS	71/80	89
HIV-negative gay men with KS	8/9	89
control tissues ^a	14/743	1.8 ^b

^a Includes high-risks patients.

^b $p < 0.0001$.

Europe, subtype A in western Europe and North America and subtype D has only so far been described in individuals from the Pacific Islands (Hayward 1999; Zong *et al.* 1999). A new subtype (E) has been described from South American indigenous people (Biggar *et al.* 2000). So far, no subtype appears to correlate with a specific disease entity or with a more aggressive course for KS. The unusually high genetic divergence identified in ORF-K1 reflects some unknown powerful biological selection process acting specifically on this immunoglobulin (Ig)-receptor-like signal-transducing protein (Hayward 1999; McGeoch & Davison 1999). This could be related to evolving mechanisms of viral evasion from the immune system among different populations. Subtypes A and C are phylogenetically closest to each other, and subtype B more distant.

Subsequent to the discovery of KSHV, several new non-human primate rhadinoviruses have been isolated which have a closer phylogenetic relationship to KSHV than either *Herpesvirus saimiri* or EBV (see Damania & Desrosiers, this issue). These new members define two distinct rhadinovirus lineages. One lineage is closely related to KSHV and includes retroperitoneal fibromatosis herpesvirus (RFHV) (Rose *et al.* 1997), *Chlorocebus* rhadinovirus (ChRV) (Greensill *et al.* 2000) and chimpanzee (PanRHV1 and PanRHV2) and gorilla rhadinoviruses (GorRHV1) (Lacoste *et al.* 2000b). The second lineage includes a rhesus monkey rhadinovirus (RRV) (Desrosiers *et al.* 1997) and ChRV2 (Greensill *et al.* 2000). The finding of two lineages of rhadinoviruses in non-human primates suggests that there might be an as yet unidentified RRV or ChRV2-type virus in humans. The possibility that KSHV was introduced from African apes to humans, rather than coevolved with *Homo sapiens*, is not excluded.

6. SERO-EPIDEMIOLOGY

(a) *Serological assays*

There are several KSHV serological assays currently available. The most widely used assays are based on the detection of latent or lytic antigens in KSHV-infected primary effusion lymphoma (PEL) cell lines, either by

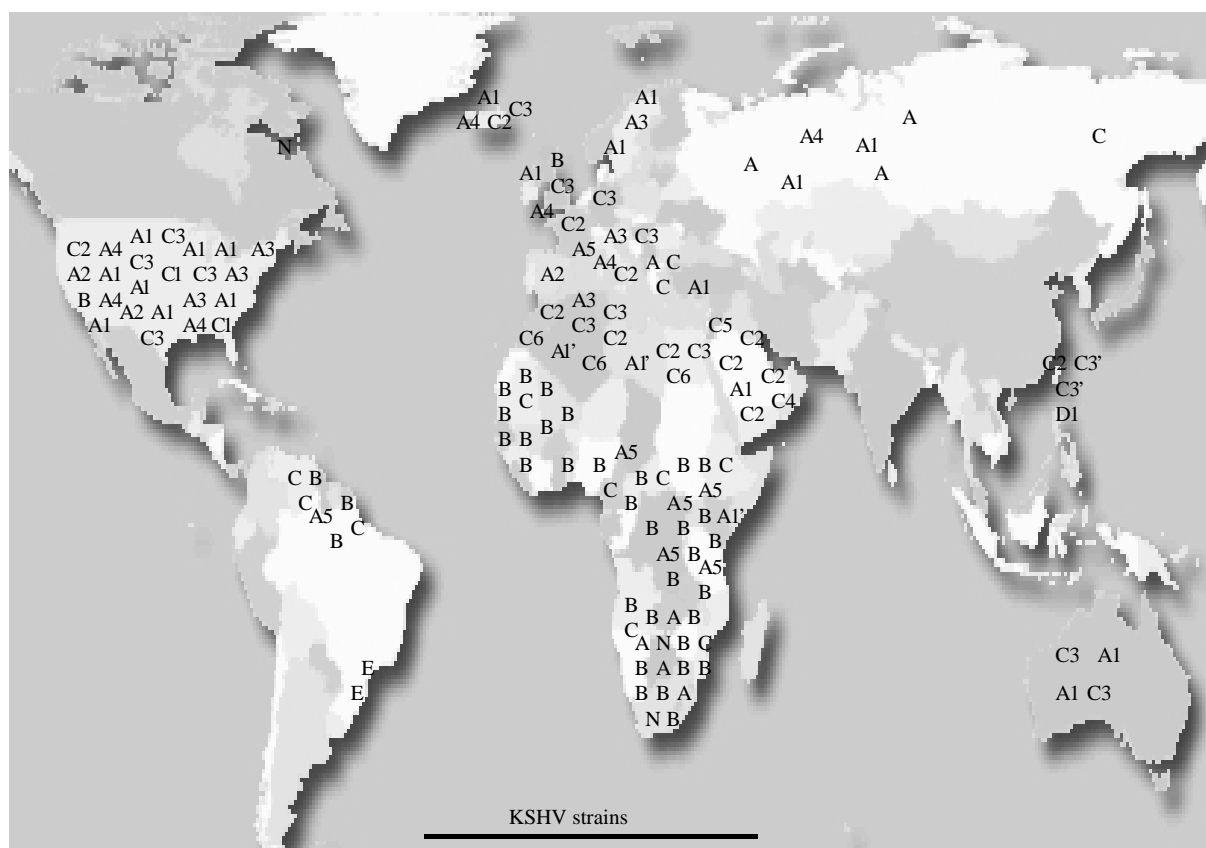


Figure 1. World distribution of KSHV strains and subtypes. The B and C subtypes described in North America are predominantly in those of African or Mediterranean origin. Data compiled from Cook *et al.* (1999), Davidovici *et al.* (2000), Hayward (1999), Lacoste *et al.* (2000a), Meng *et al.* (1999), Poole *et al.* (1999), Zong *et al.* (1999) and N. Wilder and C. Boshoff (unpublished data).

immunofluorescence (IF) (Gao *et al.* 1996a; Kedes *et al.* 1996; Simpson *et al.* 1996) or by enzyme-linked immunosorbent assay (Chatlynne *et al.* 1998). Assays have also been described which detect antibodies to recombinant KSHV latent and lytic proteins or synthetic peptides. Lytic proteins shown to be immunogenic include ORF 65 (Simpson *et al.* 1996), ORF 26 (Davis *et al.* 1997a), and ORF K8.1 (Chandran *et al.* 1998; Raab *et al.* 1998). The only latent antigen thus far to be used in recombinant assays is ORF 73, which is also the latent antigen detected in IF assays (Kedes *et al.* 1997b; Kellam *et al.* 1997; Rainbow *et al.* 1997). A study comparing various assays including recombinant proteins (ORFs 65, K8.1) and IF assays concluded that IF followed by confirmation with Western blot reactions with a panel of latent and lytic immunogenic antigens provide a reliable, sensitive and specific method to detect KSHV antibodies (Zhu *et al.* 1999).

(b) Sero-prevalence

(i) Northern Europe and North America

The sero-prevalence of KSHV in the different HIV-1 risk groups correlates with the incidence of KS: in the West, KSHV is found predominantly in HIV-positive gay men (Gao *et al.* 1996a; Kedes *et al.* 1996, 1997a; Lennette *et al.* 1996; Simpson *et al.* 1996) and not in HIV-positive heterosexuals, patients with haemophilia or intravenous drug users.

In a cohort of men in San Francisco, it was shown that KSHV infection is associated with the number of homosexual partners and correlates with a previous history of a sexually transmitted disease (e.g. gonorrhoea) and HIV infection (Martin *et al.* 1998), suggesting that KSHV is sexually transmitted. In a univariate analysis of gay men attending the sexually transmitted diseases clinic at St Thomas' Hospital in London, KSHV prevalence was associated with a history of sex with an American, suggesting that KSHV was perhaps first introduced into the gay communities in the epicentres of HIV in the USA, before spreading to Europe (Smith *et al.* 1999). In the Amsterdam gay men's cohort the risk of developing KS is higher when KSHV was acquired after HIV infection than vice versa (Renwick *et al.* 1998). This may be due to lower immune responses to primary KSHV infection in those who are HIV infected, with subsequently higher KSHV viral loads. There is also a suggestion of orogenital transmission of KSHV in this cohort (Dukers *et al.* 2000). In gay men in New York City and Washington DC the highest prevalence of KSHV infection occurred during the early 1980s and fell sharply thereafter (O'Brien *et al.* 1999).

(ii) Mediterranean Europe

The incidence of classic KS is significantly higher in Italy than in the UK or the US (Franceschi & Geddes 1995; Geddes *et al.* 1995), as is the prevalence of antibodies

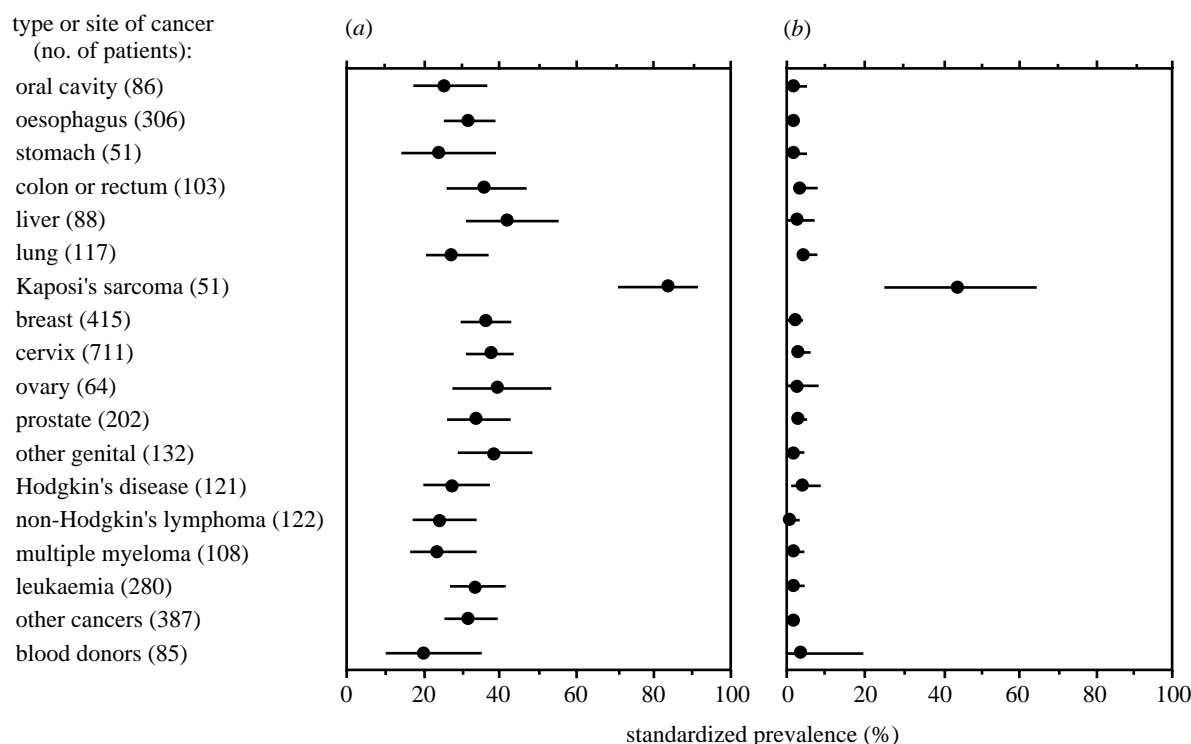


Figure 2. Antibodies to KSHV in 3400 black patients in South Africa with various types of cancer. (a) Sero-prevalence. (b) High-titre antibodies; presence of antibody titre is greater than 1:100 000. Adapted from Sitas *et al.* (1999).

to KSHV in blood donors (Cattani *et al.* 1999; Whitby *et al.* 1998). Furthermore, the incidence of classic KS in Italy shows considerable regional variation (Geddes *et al.* 1995) in parallel to KSHV prevalence (Calabro *et al.* 1998; Whitby *et al.* 1998). In addition, the geometric mean titre of anti-KSHV antibodies is highest in blood donors from the south, where the incidence of KS and the prevalence of KSHV is highest (Whitby *et al.* 1998). This finding is reminiscent of EBV infection, where high anti-EBV antibody titre correlates with an increased risk of developing Burkitt's lymphoma or nasopharyngeal carcinoma (de Thé *et al.* 1978*a,b*). The acquisition of KSHV in Italian children supports postnatal routes of transmission in families (Whitby *et al.* 2000).

(iii) Israel

Various genetic diseases appear to cluster predominantly in either Ashkenazi Jews (eastern and north-western Europe) or Sephardi Jews (North Africa and the Mediterranean). For example, Tay–Sachs syndrome is found almost exclusively among Ashkenazi Jews (Petersen *et al.* 1983), whereas familial Mediterranean fever is predominantly seen in non-Ashkenazi Jews, Arabs, Turks and Armenians (Ben-Chetrit & Levy 1998). The genetic mutations leading to these diseases would therefore appear to have been introduced after the two main arms of the diaspora separated. In contrast, the 185delAG mutation in BRCA1 was originally described as a common mutation in Ashkenazi breast and ovarian cancer families (Tonin *et al.* 1996), but has since also been reported in Sephardi, Yemeni and Iranian Jews (Bar-Sade *et al.* 1998), suggesting a mutation prior to the dispersion of the Jewish people.

Classic KS is relatively common among both Sephardi and Ashkenazi Jews and the incidence of classic KS in Israel is among the highest in the developed world (Fenig *et al.* 1998; Zahger *et al.* 1993), compared with a low age-standardized incidence in the UK and USA (Biggar *et al.* 1984; Grulich *et al.* 1992) during a similar period (table 1). North African Jews (Sephardi) have a higher incidence of classic KS than Jews born in Israel or Europe (Iscovich *et al.* 1998; Marill *et al.* 1973). Thus KSHV or a genetic predisposition to KS appears to have been introduced into the Jewish population prior to the diaspora.

The sero-prevalence of KSHV among Israeli Jews is higher than that seen in the general populations of western Europe and North America (Davidovici *et al.* 2001) and the sero-prevalence of KSHV among the different Jewish groups correlates with the incidence of KS (Davidovici *et al.*). Place of birth and sero-status of spouse are the most important risk factors for adults to be infected. Furthermore, mother-to-child transmission is important in the acquisition of KSHV in Israel (Davidovici *et al.*). A relatively high prevalence of KSHV in Egyptian children (Andreoni *et al.* 1999) indicates similar routes of transmission in other Middle Eastern countries.

(iv) Africa

While endemic KS existed in parts of Africa long before the AIDS epidemic, AIDS–KS is now the most common tumour in many parts of Africa (table 1). In sub-Saharan Africa, where KS rates are relatively high among HIV-positive individuals, the prevalence of antibodies to KSHV is also higher than in North America and northern Europe (Ariyoshi *et al.* 1998; Gao *et al.* 1996*b*; Olsen *et al.* 1998; Simpson *et al.* 1996; Sitas *et al.*

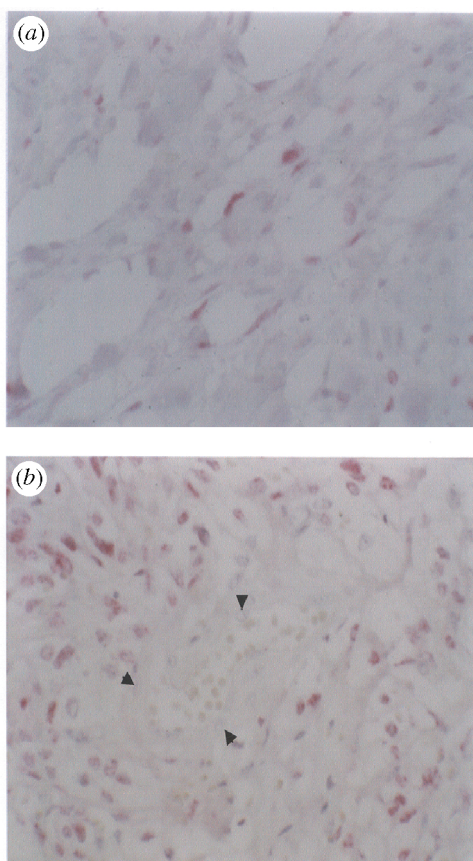


Figure 3. KSHV LANA-1 expression in early (a) and nodular (b) KS lesions. LANA-1 is only present in a minority of cells in early lesions, but in most spindle cells in advanced lesions. Normal mature endothelial cells are negative for KSHV (arrows).

1999) and KSHV infection occurred in Africa before the epidemic of AIDS-KS (de Thé *et al.* 1999). In a study of KSHV serology in over 3400 black cancer patients (Sitas *et al.* 1999), only KS was correlated with prevalence and titre of KSHV antibodies (figure 2).

Acquisition of KSHV at an early age in Africa is likely because KS is seen in African children (Ziegler & Katongole-Mbidde 1996). Indeed, the prevalence of antibodies to KSHV increases steadily with age in Africa (Olsen *et al.* 1998; Rezza *et al.* 2000; Sitas *et al.* 1999) and this occurs before puberty (Bourboulia *et al.* 1998; Gessain *et al.* 1999; He *et al.* 1998; Mayama *et al.* 1998). This indicates that KSHV is not predominantly transmitted during sex, as with gay men in the West. Mother-to-child transmission and sibling-to-sibling transmission has been shown to occur in South Africa and in an Afro-Caribbean population living in French Guyana (Bourboulia *et al.* 1998; Planoulaine *et al.* 2000). About one-third of KSHV-positive black mothers in South Africa transmit the virus to their children (Bourboulia *et al.* 1998). In South Africa there is significantly lower prevalence of anti-KSHV antibodies among whites than among blacks; in black cancer patients the sero-prevalence of KSHV declines with increasing education, suggesting that factors associated with poverty may contribute to the transmission of the virus (Sitas *et al.* 1999).

The lymph nodal form of KS seen in African children might be a manifestation of primary infection by KSHV,

analogous to EBV-associated infectious mononucleosis. In support of this notion is the description of microscopical KS-type changes in the lymph node of an HIV-infected 43-year-old French patient at the time of presumed KSHV sero-conversion (Oksenhendler *et al.* 1998).

7. TRANSMISSION

Although one group reported the frequent detection of KSHV in the semen of healthy Italian donors (Monini *et al.* 1996a), in North America and the UK the current consensus is that KSHV is present only intermittently in the semen of patients with KS and sometimes in HIV-positive patients without KS, but only rarely in semen donors (Ambroziak *et al.* 1995; Corbellino *et al.* 1996a; Howard *et al.* 1997; Lin *et al.* 1998; Tasaka *et al.* 1996; Viviano *et al.* 1997). KSHV has also been reported in prostate biopsies of HIV-positive men with and without KS (Diamond *et al.* 1998; Staskus *et al.* 1997), so KSHV shedding into semen from prostate fluid is therefore a possible mode of transmission. Infectious virus is also found in the saliva of HIV-positive individuals (Koelle *et al.* 1997). In patients with classic KS, KSHV DNA was found in tonsillar swabs and in saliva (Cattani *et al.* 1999). Although studies in gay men indicate that KSHV is transmitted during sex and the risk of having KSHV increases with the number of sexual partners, the exact mode and route of transmission is not known. Semen and saliva are each possible routes of viral transmission, but their respective contribution to infection is unknown. The role of breast milk, saliva and other transmission routes for mother-to-child and sibling-to-sibling transmission is also still unknown.

8. KAPOSI'S SARCOMA AND KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS INFECTION

Four observations link KSHV causally to the aetio-pathogenesis of KS, although none of these findings on their own is sufficient to support a causative role:

- (i) KSHV DNA is present, by PCR, in all epidemiological forms of KS, in all fresh biopsies tested and in the vast majority of paraffin-embedded material. However, KSHV DNA is rarely, if at all, detectable in other mesenchymal tumours (IARC 1997) (table 2).
- (ii) The detection of KSHV DNA by PCR in the peripheral blood of HIV-positive individuals predicts who might subsequently develop KS (Moore *et al.* 1996; Whitby *et al.* 1995), indicating that those at risk of KS have a higher viral load than those not at risk.
- (iii) Serological studies indicate that in general populations at risk of developing KS there is a higher prevalence of KSHV infection, so that the incidence of classic KS and AIDS-KS in different populations correlates broadly with the prevalence of virus infection in these populations.
- (iv) In advanced KS lesions, KSHV is latently expressed in nearly all the tumour (spindle) cells (Dupin *et al.* 1999; Sturzl *et al.* 1999). This is reminiscent of other viral cancers, e.g. EBV latent infection in post-transplant lymphoproliferative disease or HPV infection in cervical cancer.

To strengthen the molecular epidemiological association between KSHV and KS further, it was demonstrated by PCR *in situ* hybridization, RNA *in situ* hybridization and immunohistochemistry that KSHV is present in spindle cells in nearly all KS lesions (Boshoff *et al.* 1995a; Davis *et al.* 1997b; Dupin *et al.* 1999; Kellam *et al.* 1999; Li *et al.* 1996; Rainbow *et al.* 1997; Staskus *et al.* 1997; Sturzl *et al.* 1997) (figure 3). In early KS lesions, only a small proportion (<10%) of spindle cells are positive for KSHV, whereas VEGFR-3 is expressed by most cells (Dupin *et al.* 1999), indicating that paracrine mechanisms may be important in the initiation and progression of KS. In nodular lesions, more than 90% of the spindle cells contain KSHV latent infection, suggesting that KSHV latent proteins confer a growth advantage on infected cells (Dupin *et al.* 1999). KSHV is not present in other vascular tumours, including angiomas and angiosarcomas, and it is only rarely detectable in other forms of skin tumours (including squamous carcinomas and melanomas) in immunosuppressed patients (Adams *et al.* 1995; Boshoff *et al.* 1996; Lin *et al.* 1996; Uthman *et al.* 1996).

In culture, KSHV has been shown to infect macro- and microvascular endothelium and to provide a growth advantage to these cells (Flore *et al.* 1998). With other oncoproteins like HPV E6 and E7, KSHV can transform human microvascular endothelium (Moses *et al.* 1999). Our current model is that KSHV, like EBV, persists in B cells as an episome expressing only a small number of latent proteins. Reactivation of the virus due to immunosuppression or local cytokine production leads to infection of endothelial precursors. In these precursors, KSHV latent proteins induce proliferation and block differentiation and apoptosis by anti-viral cellular immune responses. It is possible that local cytokine production stimulates neo-angiogenesis or neo-lymphangiogenesis and KSHV then infects these immature endothelial cells.

There is currently no evidence that KSHV infects endothelial cells in healthy individuals. Although the nature of the spindle cells in KS has been studied extensively there remains uncertainty regarding their exact origin. These cells usually express proteins that are found exclusively on endothelial cells. More recently, VEGFR-3/*Flt-4* was shown to be expressed by all the KSHV-positive spindle cells in KS lesions. VEGFR-3 is the receptor for the angiopromoting cytokines VEGF-C and VEGF-D. As VEGF-C promotes lymphangiogenesis and VEGFR-3 is highly expressed by mature lymphatic endothelia we propose that the KS tumour cell belongs to the lymphatic endothelium lineage of cells (precursors), rather than being de-differentiated mature microvascular endothelium (Dupin *et al.* 1999). The expression by all KS spindle cells of another marker related to lymphatic endothelium, podoplanin, supports this hypothesis (Weninger *et al.* 1999). *In vitro*, VEGF-C promotes a mitogenic and motogenic response of KS-derived spindle cells by activating the tyrosine phosphorylation of VEGFR-3 (Marchio *et al.* 1999).

The major viral latency-associated nuclear antigen (LANA-1 or LNA-1) encoded by ORF 73 appears to be expressed in virtually all KSHV-infected cells (Dupin *et al.* 1999; Parravicini *et al.* 2000; Katano *et al.* 2000). Using an antibody against LANA-1, Dupin *et al.* (1999) detected KSHV in nearly all spindle cells of well-developed KS

lesions, but in only a few endothelial or spindle cells of early KS lesions, suggesting that paracrine mechanisms might be involved in the initiation of these lesions.

The histological, clinical and molecular features may be reconciled by invoking a model for KS in which early lesions are non-clonal proliferations of virus-infected endothelial or endothelial precursors and advanced disease represents oligoclonal or monoclonal neoplasms. This model is comparable with EBV-driven polyclonal lymphoproliferative disorders in immunodeficient individuals, which can progress to clonal lymphomas. The contribution to pathogenesis of lytically replicating virus in a subset of KS spindle cells has not been determined, although various lytic KSHV proteins trigger, at least in experimental systems, pathways involved in cell proliferation, anti-apoptosis, cell migration and angiogenesis (see Moore & Chang, this issue). In early lesions such a paracrine model could be involved in the pathogenesis of KS.

9. KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS AND LYMPHOPROLIFERATION

(a) *Body cavity-based primary effusion lymphoma*

Two groups first recognized the unique aspects of some effusion-based lymphomas in patients with AIDS (Knowles *et al.* 1989; Walts *et al.* 1990). The cells in these cases were negative for most lineage-associated antigens, although a B-cell origin was indicated with clonal rearrangement of the Ig genes. Karcher *et al.* (1992) further demonstrated the distinctiveness of the syndrome, reporting a high prevalence of EBV and absence of *c-myc* rearrangements. Later, KSHV was specifically associated with primary effusion lymphoma (PEL), but not with any of the common types of Hodgkin's and non-Hodgkin's lymphomas (Cesarman *et al.* 1995; Gessain *et al.* 1997; Sitas *et al.* 1999).

PEL typically presents as malignant effusions in the visceral cavities usually without significant tumour mass or lymphadenopathy. These lymphomas occur predominantly in HIV-infected individuals with advanced stages of immunosuppression (Komanduri *et al.* 1996), but are occasionally seen in HIV-sero-negative patients (Nador *et al.* 1996; Strauchen 1997; Said 1996). As with KS, PELs are seen primarily in gay men (Jaffe 1996; Nador *et al.* 1996). PELs usually do not express surface B-cell antigens with the exception of CD138/syndecan-1, a molecule selectively associated with late stages of B-cell differentiation (Gaidano *et al.* 1999). This finding, and frequent mutations in the 5' non-coding region of BCL-6, define PEL cells as preterminally differentiated, post-germinal-centre stage B cells (Gaidano *et al.* 1999) (table 3). It appears that KSHV-positive PEL cells lack many adhesion molecules and 'homing markers' present on other diffuse lymphomas: this may contribute to the peculiar effusion phenotype of these lymphomas and the usual lack of macroscopic lymph nodal involvement (Boshoff *et al.* 1998).

PEL cells consistently lack molecular defects commonly associated with neoplasia of mature B cells including activation of the proto-oncogenes *c-myc*, *bcl-2*, *bcl-6*, *n-ras*, and *k-ras*, as well as mutations of p53 (Nador *et al.* 1996). Southern blot analysis of PEL cells shows the presence of the KSHV genome in very high

Table 3. Comparison of KSHV-infected PEL cells and plasmablasts in multicentric Castleman's disease

(The term plasmablast is used to connote a medium-sized cell with a moderate amount of amphophilic cytoplasm and a large vesicular nucleus containing up to three prominent nucleoli. In contrast to an immunoblast, the cytoplasm contains abundant Ig (Dupin 2000).)

feature	PEL	plasmablastic multicentric Castleman's disease
site	body cavity, extranodal	lymph nodes, spleen
morphology	immunoblastic	plasmablastic
KSHV	positive	positive
EBV	positive in majority	negative
cytoplasmic Ig expression	absent	high level of IgM
Ig light chain	monotypic κ or λ mRNA	monotypic λ Ig
CD30	positive	weakly positive
CD138	positive	not determined
B-cell antigens	absent	weak or absent
mutation in Ig genes	hypermuted in majority	absent
cellular origin	post-germinal-centre B cells	naive IgM λ -expressing B cells

copy number (50–150 cell⁻¹) compared with that seen in KS. Cell lines from PEL have been established (Arvanitakis *et al.* 1996; Cesarman *et al.* 1995; Renne *et al.* 1996). Some cell lines are only positive for KSHV; others are co-infected with EBV. In addition to cell lines established from lymphomatous effusions, the BCP-1 cell line was established from the peripheral blood of a patient with PEL suggesting that the malignant cells are present not only in the malignant effusions but may be also found disseminated in the peripheral blood (Boshoff *et al.* 1998). PEL cell lines express KSHV latent genes and can readily be induced by phorbol esters or butyrate into lytic replication (see Moore & Chang, this issue). Thus PEL cells have been of great use in defining KSHV gene expression and for the production of antigens, as well as for IF assay substrates (Gao *et al.* 1996b; Jenner *et al.* 2001; Kedes *et al.* 1996; Lennette *et al.* 1996).

A PEL-derived cell line, BC-3, was used to study the global expression of all known KSHV genes using gene expression microarray technology (figure 4). Cluster analysis, which arranges genes according to their expression profile, revealed a correlation between expression and assigned gene function that is consistent with the known stages of herpesvirus life cycles (Jenner *et al.* 2001). This study showed that non-induced PEL cells express a highly restricted number of KSHV genes (figure 4b) and these genes are presumably involved in maintaining the proliferation of these cells (see Moore & Chang, this issue).

KSHV has not yet been shown to transform or immortalize any B-cell type *in vitro*. However, the target cells used (i.e. mature B lymphocytes) may not be those that are susceptible to KSHV infection.

(b) Castleman's disease

Multicentric Castleman's disease (MCD) is a lymphoproliferative disorder of unknown aetiology and is associated with the development of secondary B-cell lymphoma (Castleman *et al.* 1956; Frizzera 1988). We now know that a subgroup of MCD is linked to KSHV. KSHV infection is found in nearly 100% of HIV-associated cases of MCD and 40–50% of HIV-negative cases (Chadburn *et al.* 1997; Corbellino *et al.* 1996b; Gessain *et al.* 1996; Soulier *et al.* 1995). HIV-sero-negative

patients with KSHV-related MCD appear to experience a worse clinical course than KSHV-negative MCD and their disease is frequently complicated by autoimmune haemolytic anaemia and polyclonal gammopathies (Chadburn *et al.* 1997; Parravicini *et al.* 1997).

In MCD, KSHV is present in plasmablasts belonging to the B-cell lineage, which are not present in KSHV-negative MCD (Dupin *et al.* 1999; Katano *et al.* 2000). These plasmablasts localize mainly in the mantle zone of B-cell follicles. KSHV-positive MCD is therefore a distinct disease entity and designated as a plasmablastic variant of MCD (Dupin *et al.* 2000). Confluent clusters of KSHV-positive plasmablasts are also present in biopsies of plasmablastic MCD, indicating that isolated KSHV plasmablasts can progress to form foci of microlymphoma. KSHV is also present in all the tumour cells of plasmablastic lymphomas that develop in patients with the KSHV-positive plasmablastic variant of MCD (Dupin *et al.* 2000). The development of plasmablastic lymphoma therefore appears to represent a further evolution of this disorder (figure 5). Unlike KSHV-positive PEL cells, the plasmablasts in MCD are only positive for KSHV, and not for EBV (table 3).

The KSHV-positive plasmablasts express high levels of cytoplasmic IgM and show exclusively λ light chain restriction. In contrast, KSHV-negative, mature plasma cells in the interfollicular region, a prominent feature of MCD, are IgM negative and usually polyclonal. KSHV-positive plasmablasts in MCD typically occur as isolated cells but may coalesce to form microscopic aggregates that show λ light chain restriction and therefore have been referred to as microlymphomas (Dupin *et al.* 2000). In some cases, KSHV-positive plasmablasts may form frank plasmablastic lymphoma. In all lesions in 25 patients studied so far, KSHV-positive plasmablasts uniformly express IgM λ (Dupin *et al.* 2000).

The finding that KSHV-positive plasmablasts in MCD are monotypic raises the question whether this is indicative of monoclonality or of preferential targeting of λ -expressing B cells by the virus (Dupin *et al.* 2000). It was recently shown that these monotypic cells are polyclonal as determined by Ig gene rearrangement (Du *et al.* 2001). KSHV infection therefore invokes a monotypic but

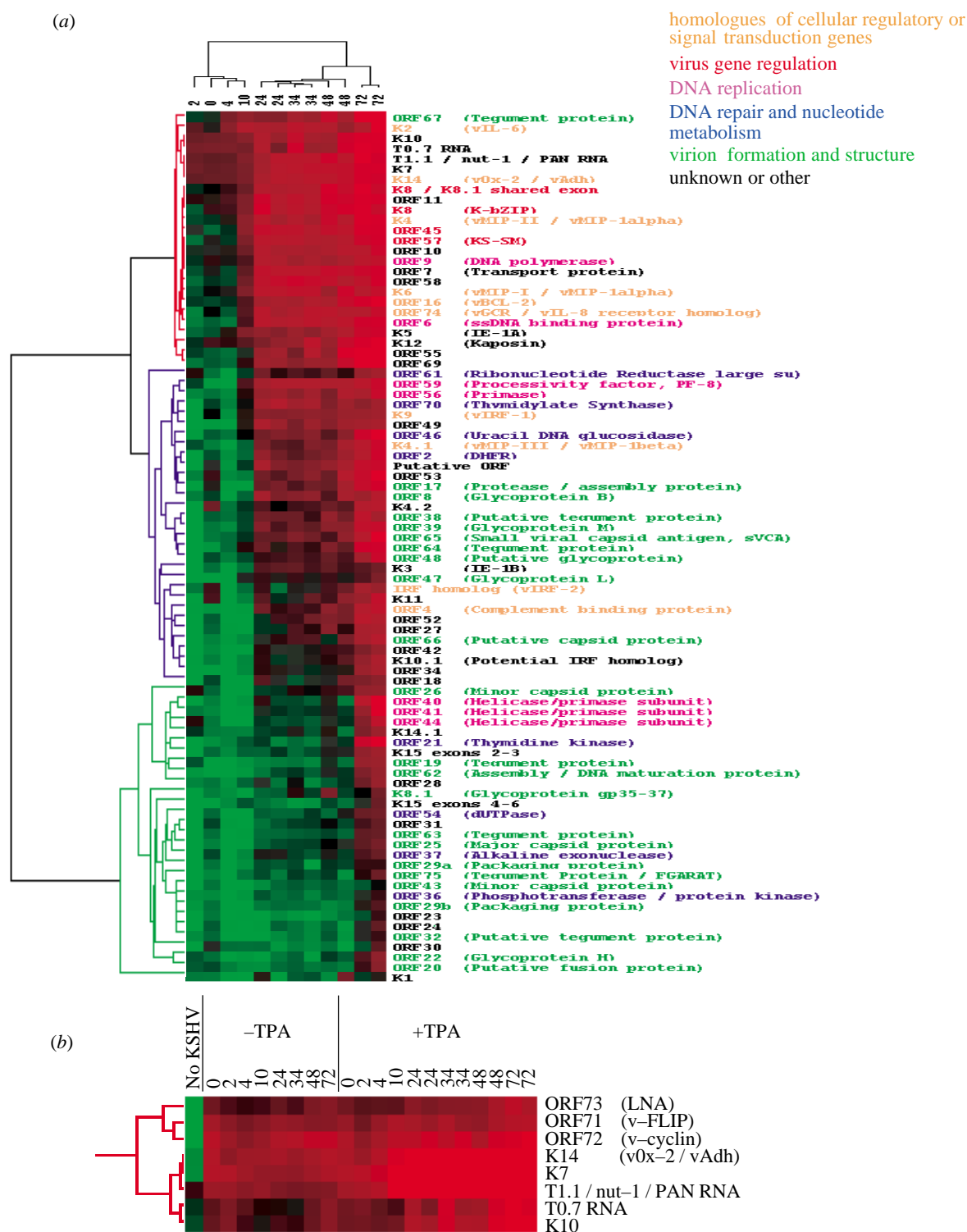


Figure 4. KSHV gene expression as determined by array technology and hierarchical clustering (from Jenner *et al.* 2001).

(a) Hierarchical clustering of the genes and samples after the induction of lytic replication with TPA. The ORF and corresponding gene names are listed on the right-hand side and are colour-coded according to their putative function.

(b) Expanded view of the cluster of genes whose expression is detectable in non-induced PEL cells.

polyclonal B-cell proliferation in MCD and in some cases KSHV-positive plasmablasts may form polyclonal or monoclonal microlymphomas or develop into frank monoclonal lymphoma (figure 5). These events (polyclonal to monoclonal evolution) are similar to those in

lymphoproliferative disorders caused by EBV in immuno-suppressed hosts. However, EBV-associated lymphoproliferative disease, unlike KSHV-related lymphoproliferative disorders in MCD patients, tends to be of germinal-centre or post-germinal-centre origin.

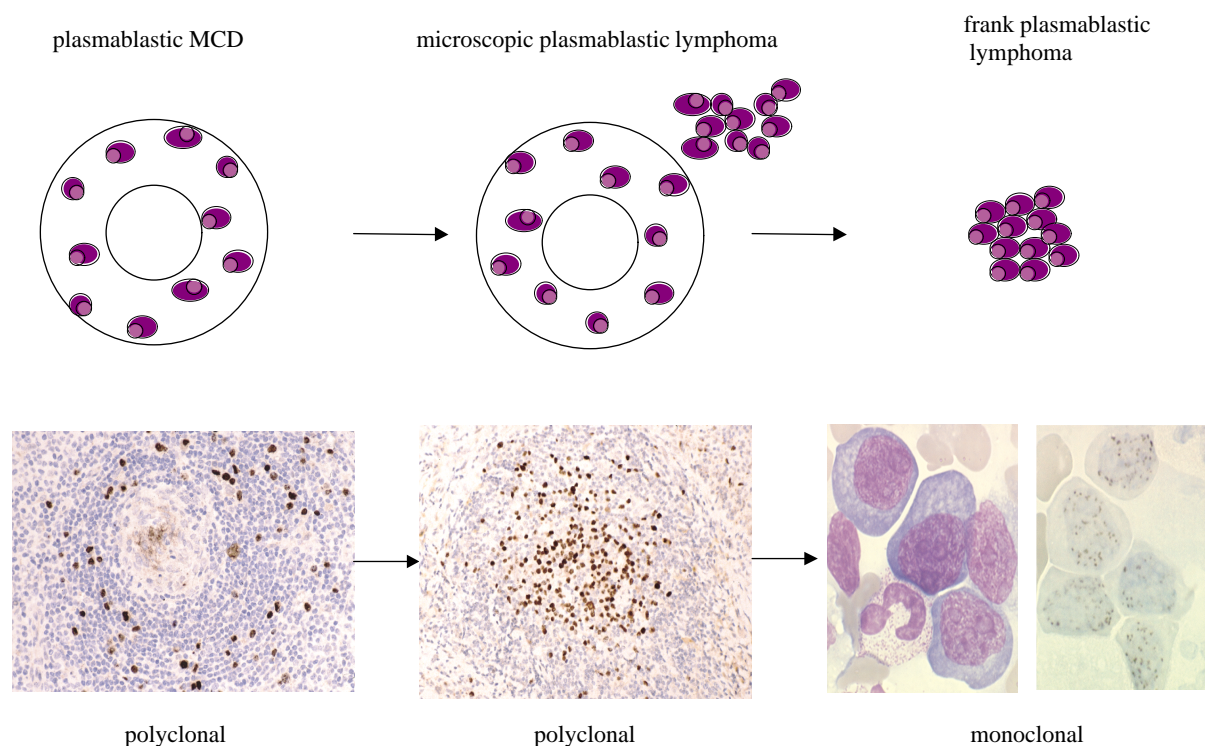


Figure 5. Model of KSHV in plasmablastic MCD. LANA-1 staining is shown in the bottom panel.

The reasons why KSHV is overwhelmingly associated with polyclonal λ light-chain-expressing B cells in MCD are not clear. In PEL, the tumour cells express functionally rearranged κ or λ light chain transcripts. In MCD, occasional KSHV-positive κ -expressing B cells are also seen. KSHV may naturally target both κ and λ light-chain-expressing B cells without bias, but λ cells may expand preferentially due to their intrinsic proliferative response to KSHV infection. The finding of κ light-chain-positive PEL does not argue against this hypothesis as the majority of PEL are also co-infected with EBV, which might override the differential response of κ and λ light-chain-expressing B cells to KSHV infection.

KSHV-positive plasmablasts do not harbour somatic mutations in the rearranged Ig gene, indicating that they originate from naive B cells despite their mature phenotype (table 3). These findings are consistent with their preferential localization in the mantle zones of B-cell follicles in MCD, and the lack of detectable follicular dendritic cell meshwork within the microlymphoma sites even though they are often adjacent to or partially replace B-cell follicles. Thus, KSHV may infect IgM⁺ naive B cells and drive these cells to differentiate into plasmablasts without undergoing the usual germinal-centre reaction, during which normal naive B cells mutate their rearranged Ig genes and differentiate into plasma or memory B cells. Like the plasmablasts in MCD, the plasmablastic lymphomas associated with MCD harbour non-mutated Ig genes and are derived from naive B cells. Two or even three independent KSHV-associated tumours (i.e. KS, PEL and MCD) in the same individual have been reported (Codish *et al.* 2000; Jones *et al.* 1998).

Viral IL-6 (vIL-6) is highly expressed in 10–15% of KSHV-positive plasmablasts (Du *et al.* 2001; Parravicini *et al.* 1998), and the IL-6 receptor is strongly expressed in the majority of KSHV-positive cells. Activation of the IL-6 signalling pathway may play an important role in driving KSHV-infected naive B cells to differentiate into plasmablasts and to develop various lymphoproliferative lesions. vIL-6 may directly stimulate both vIL-6-positive and -negative cells infected with KSHV by autocrine and paracrine mechanisms (see Moore & Chang, this issue). Elevated serum human IL-6 (hIL-6) has been demonstrated in patients with MCD and the level of hIL-6 correlated with the clinical presentation of the disease and high KSHV viral load in PBMC. Overproduction of hIL-6 is thought to be responsible for the systemic manifestation of MCD as blockage of IL-6 signalling by antibody can dramatically alleviate both clinical and histological presentations of the disease. Although it remains to be tested whether the elevated hIL-6 in MCD is the result of KSHV infection, it is noteworthy that hIL-6 is produced by PEL and promotes the growth of PEL cells *in vitro* and *in vivo*.

A role for HIV-1 in the pathogenesis of KS is supported by the observations that KS can rapidly resolve with highly active anti-retroviral therapy (HAART). Whether these responses are purely due to a restoration of cellular immunity against KSHV or because of the lower circulating HIV-1 load, is not yet known. In contrast, KSHV-positive MCD does not often resolve and can, despite HAART, progress to fatal lymphoma (Dupin *et al.* 2000; Zietz *et al.* 1999).

ORF 73 encodes LANA-1 of KSHV (Kedes *et al.* 1997b; Kellam *et al.* 1997; Rainbow *et al.* 1997). LANA-1 is expressed in all tumour cells in each of the KSHV-related

Table 4. *Convergent evolution in the functions of certain DNA tumour viral nuclear phosphoproteins*

(Minus and plus signs denote does not bind to and binds to, respectively.)

	SV40 large T antigen	papillomavirus		KSHV LANA-1	adenovirus	
		E6	E7		E1A	E1B
binds to pRB	+	—	—	+	+	—
binds to p53	+	+	—	+	—	+
binds to histone H1	?	—	+	+	?	?
maintains episome	?	+	+	+	+	—
transform primary rodent cells with a cellular oncogene	+	+	+	+	+	+

malignancies (figures 3 and 5). LANA-1 has been shown to maintain the KSHV episome, and tethers the viral genome to chromatin during mitosis via histone H1 (Ballestas *et al.* 1999; Cotter & Robertson 1999). LANA-1 interacts with the tumour-suppressor protein p53 and represses its transcriptional activity (Friborg *et al.* 1999). LANA-1 also binds to RING3 (Platt *et al.* 1999) and pRB (Radkov *et al.* 2000), two proteins involved in regulating E2F-dependent transcription. It is notable that other DNA tumour viral proteins that tether viral DNA to chromatin during mitosis also interfere with the p53 and pRb pathways (table 4).

10. CONCLUSIONS

Epidemiological evidence is overwhelming in support of KSHV as the central factor in the development of KS, the most common AIDS-related malignancy. The complex histology and expression pattern of KSHV proteins suggests that the role of the virus in KS pathogenesis is not straightforward and the model of KS tumorigenesis might not be like any other virally induced malignancy (Gallo 1998; Ganem 1996). The cell biology of KSHV also supports its role in the pathogenesis of a sub-type of MCD and of PEL.

The array of genes encoded by KSHV, some of which are unique to KSHV and others of which are shared only among the rhadinoviruses, has provided clues and directions in dissecting out the mechanisms of viral pathogenesis and oncogenesis. Investigation of these genes has begun to demonstrate their functional activity in cellular signalling and regulatory pathways (see Moore & Chang, this issue). Candidate oncogenes have been identified which may cause dysregulated proliferation or interfere with established tumour-suppressor pathways.

Like EBV, KSHV probably establishes a persistent infection which is normally controlled by the immune system and the number of KSHV-infected cells is under immunological control. When this immune control declines due to acquired or iatrogenic immunosuppression, the number of KSHV-infected cells increases with subsequent unchecked proliferation of virally infected cells and development of KSHV-related tumours. The introduction of HAART has led to a decline in the incidence of KS in AIDS patients and also to the resolution of KS in those already affected. This suggests that cellular immune responses, compromised in AIDS, but recovering after HAART, could be important in the control of

KSHV infection and in the development of KS. Remission of KS is also seen in organ-transplant patients upon cessation of immunosuppressive therapy. The rapid resolution of KS in some HIV-positive patients commencing HAART suggests that a small improvement in immunity might be important in disease control.

REFERENCES

- Adams, V., Kempf, W., Schmid, M., Muller, B., Briner, J. & Burg, G. 1995 Absence of herpesvirus-like DNA sequences in skin cancers of non-immunosuppressed patients. *Lancet* **346**, 1715–1716.
- Albini, A., Barillari, G., Benelli, R., Gallo, R. C. & Ensoli, B. 1995 Angiogenic properties of human immunodeficiency virus type 1 Tat protein. *Proc. Natl Acad. Sci. USA* **92**, 4838–4842.
- Albini, A., Benelli, R., Presta, M., Rusnati, M., Ziche, M., Rubartelli, A., Pagliarunga, G., Bussolino, F. & Noonan, D. 1996 HIV-Tat protein is a heparin-binding angiogenic growth factor. *Oncogene* **12**, 289–297.
- Albini, A. (and 10 others) 1997 The angiogenesis induced by HIV-1 Tat protein is mediated by the Flk-1/KDR receptor on vascular endothelial cells. *Nat. Med.* **2**, 1371–1375.
- Ambroziak, J. A., Blackburn, D. J., Herndier, B. G., Glogau, R. G., Gullett, J. H., McDonald, A. R., Lennette, E. T. & Levy, J. A. 1995 Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. *Science* **268**, 582–583.
- Andreoni, M., El-Sawaf, G., Rezza, G., Ensoli, B., Nicastri, E., Ventura, L., Ercoli, L., Sarmati, L. & Rocchi, G. 1999 High seroprevalence of antibodies to human herpesvirus-8 in Egyptian children: evidence of nonsexual transmission. *J. Natl Cancer Inst.* **91**, 465–469.
- Ariyoshi, K. (and 11 others) 1998 Kaposi's sarcoma in the Gambia, west Africa is less frequent in human immunodeficiency virus type 2 than in human immunodeficiency virus type 1 infection despite a high prevalence of human herpesvirus 8. *J. Hum. Virol.* **1**, 192–199.
- Arvanitakis, L., Mesri, E. A., Nador, R. G., Said, J. W., Asch, A. S., Knowles, D. M. & Cesarman, E. 1996 Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus. *Blood* **88**, 2648–2654.
- Asahara, T., Murohara, T., Sullivan, A., Silver, M., Van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G. & Isner, J. M. 1997 Isolation of putative endothelial cells for angiogenesis. *Science* **275**, 964–967.
- Ballestas, M. E., Chatis, P. A. & Kaye, K. M. 1999 Efficient persistence of extrachromosomal KSHV DNA mediated by latency-associated nuclear antigen. *Science* **284**, 641–644.
- Barnett, S. W., Murthy, K. K., Herndier, B. G. & Levy, J. A. 1994 An AIDS-like condition induced in baboons by HIV-2. *Science* **266**, 642–646.

- Bar-Sade, R. B. (and 11 others) 1998 The 185delAG BRAC1 mutation originated before the dispersion of Jews in the diaspora and is not limited to Ashkenazim. *Hum. Mol. Genet.* **7**, 801–805.
- Bassett, M. T., Chokunonga, E., Mauchaza, B., Levy, L., Ferlay, J. & Parkin, D. M. 1995 Cancer in the African population of Harare, Zimbabwe, 1990–1992. *Int. J. Cancer* **63**, 29–36.
- Ben-Chetrit, E. & Levy, M. 1998 Familial Mediterranean fever. *Lancet* **351**, 659–664.
- Beral, V., Peterman, T. A., Berkelman, R. L. & Jaffe, H. W. 1990 Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? *Lancet* **335**, 123–128.
- Bertaccini, G. 1959 Reticulosarcoma insorto su precedente tipica sarcomatosi di Kaposi. *Dermatologia* **10**, 161.
- Biggar, R., Horm, J., Fraumeni, J., Greene, M. & Goedert, J. 1984 Incidence of Kaposi's sarcoma and mycosis fungoides in the United States including Puerto Rico, 1973–81. *J. Natl Cancer Inst.* **73**, 89–94.
- Biggar, R. J., Whitby, D., Marshall, V., Linhares, A. C. & Black, F. 2000 Human herpesvirus 8 in Brazilian Amerindians: a hyperendemic population with a new subtype. *J. Infect. Dis.* **181**, 1562–1568.
- Boshoff, C., Schulz, T. F., Kennedy, M. M., Graham, A. K., Fisher, C., Thomas, A., McGee, J. O., Weiss, R. A. & O'Leary, J. J. 1995a Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. *Nat. Med.* **1**, 1274–1278.
- Boshoff, C., Whitby, D., Hatzioannou, T., Fisher, C., Van der Walt, J., Hatzakis, A., Weiss, R. & Schulz, T. 1995b Kaposi's sarcoma-associated herpesvirus in HIV-negative Kaposi's sarcoma. *Lancet* **345**, 1043–1044.
- Boshoff, C., Talbot, S., Kennedy, M., O'Leary, J., Schulz, T. & Chang, Y. 1996 HHV8 and skin cancers in immunosuppressed patients. *Lancet* **347**, 338–339. [Erratum, **348**, 138.]
- Boshoff, C. (and 11 others) 1998 Establishment of a KSHV positive cell line (BCP-1) from peripheral blood and characterizing its growth *in vivo*. *Blood* **91**, 1671–1679.
- Bourboulia, D., Whitby, D., Boshoff, C., Carrara, H., Newton, R., Beral, V. & Sitas, F. 1998 Serological evidence for vertical transmission of KSHV/HHV-8 in healthy South African children. *J. Am. Med. Assoc.* **280**, 31–32.
- Browning, P. J., Sechler, J. M., Kaplan, M., Washington, R. H., Gendelman, R., Yarchoan, R., Ensoli, B. & Gallo, R. C. 1994 Identification and culture of Kaposi's sarcoma-like spindle cells from the peripheral blood of human immunodeficiency virus-1-infected individuals and normal controls. *Blood* **84**, 2711–2720.
- Buonaguro, F. M., Tornesello, M. L., Beth-Giraldo, E., Hatzakis, A., Mueller, N., Downing, R., Biryamwaho, B., Sempala, S. D. & Giraldo, D. 1996 Herpesvirus-like DNA sequences detected in endemic, classic, iatrogenic and epidemic Kaposi's sarcoma (KS) biopsies. *Int. J. Cancer* **65**, 25–28.
- Calabro, M. L., Sheldon, J., Favero, A., Simpson, G. R., Fiore, J. R., Gomes, E., Angarano, G., Chieco-Bianchi, L. & Schulz, T. F. 1998 Seroprevalence of Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 in several regions in Italy. *J. Hum. Virol.* **1** 207–213.
- Castleman, B., Iverson, L. & Menendez, V. P. 1956 Localized mediastinal lymph-node hyperplasia resembling thymoma. *Cancer* **9**, 822–830.
- Cathomas, G., McGandy, C. E., Terracciano, L. M., Itin, P. H., De Rosa, G. & Gudat, F. 1996 Detection of herpesvirus-like DNA by nested PCR on archival skin biopsy specimens of various forms of Kaposi sarcoma. *J. Clin. Pathol.* **49**, 631–633.
- Cattani, P., Capuano, M., Cerimele, F., La Parola, I. L., Santangelo, R., Masini, C., Cerimele, D. & Fadda, G. 1999 Human herpesvirus 8 seroprevalence and evaluation of nonsexual transmission routes by detection of DNA in clinical specimens from human immunodeficiency virus-seronegative patients from central and southern Italy, with and without Kaposi's sarcoma. *J. Clin. Microbiol.* **37**, 1150–1153.
- Cesarman, E., Chang, Y., Moore, P. S., Said, J. W. & Knowles, D. M. 1995 Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *New Engl. J. Med.* **332**, 1186–1191.
- Chadburn, A., Cesarman, E., Nador, R. G., Liu, Y. F. & Knowles, D. M. 1997 Kaposi's sarcoma-associated herpesvirus sequences in benign lymphoid proliferations not associated with human immunodeficiency virus. *Cancer* **80**, 788–797.
- Chandran, B., Smith, M. S., Koelle, D. M., Corey, L., Horvat, R. & Goldstein, E. 1998 Reactivities of human sera with human herpesvirus-8 infected BCBL-1 cells and identification of HHV-8-specific proteins and glycoproteins and the encoding cDNAs. *Virology* **243** 208–217.
- Chang, Y., Cesarman, E., Pessin, M. S., Lee, F., Culpepper, J., Knowles, D. M. & Moore, P. S. 1994 Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* **266**, 1865–1869.
- Chang, Y. (and 10 others) 1996 Kaposi's sarcoma-associated herpesvirus and Kaposi's sarcoma in Africa. *Arch. Int. Med.* **156**, 202–204.
- Chatlynne, L. G. (and 12 others) 1998 Detection and titration of human herpesvirus-8-specific antibodies in sera from blood donors, acquired immunodeficiency syndrome patients, and Kaposi's sarcoma patients using a whole virus enzyme-linked immunosorbent assay. *Blood* **92**, 53–58.
- Chuck, S., Grant, R. M., Katongole-Mbidde, E., Conant, M. & Ganem, D. 1996 Frequent presence of a novel herpesvirus genome in lesions of human immunodeficiency virus-negative Kaposi's sarcoma. *J. Infect. Dis.* **173**, 248–251.
- Codish, S., Abu-Shakra, M., Ariad, S., Zirkin, H. J., Yermiyahu, T., Dupin, N., Boshoff, C. & Suenik, S. 2000 Manifestations of three HHV-8 related diseases in an HIV-negative patient: immunoblastic variant multicentric Castleman's disease, primary effusion lymphoma, and Kaposi's sarcoma. *Am. J. Hematol.* **65**, 310–314.
- Cook, P. M., Whitby, D., Calabro, M. L., Luppi, M., Kakoola, D. N., Hjalgrim, H., Ariyoshi, K., Ensoli, B., Davison, A. J. & Schulz, T. F. 1999 Variability and evolution of Kaposi's sarcoma-associated herpesvirus in Europe and Africa. International collaborative group. *AIDS* **13**, 1165–1176.
- Corbellino, M., Bestetti, G., Galli, M. & Parravicini, C. 1996a Absence of HHV-8 in prostate and semen. *New Engl. J. Med.* **335**, 1237.
- Corbellino, M., Poirel, L., Aubin, J. T., Paulli, M., Magrini, U., Bestetti, G., Galli, M. & Parravicini, C. 1996b The role of human herpesvirus 8 and Epstein-Barr virus in the pathogenesis of giant lymph node hyperplasia (Castleman's disease). *Clin. Infect. Dis.* **22**, 1120–1121.
- Cotter, M. A. & Robertson, E. S. 1999 The latency-associated nuclear antigen tethers the Kaposi's sarcoma-associated herpesvirus genome to host chromosomes in body-cavity-based lymphoma cells. *Virology* **264**, 254–264.
- Cottoni, F., De Marco, R. & Montesu, M. A. 1996 Classical Kaposi's sarcoma in north-east Sardinia: an overview from 1977 to 1991. *Br. J. Cancer* **72**, 1132–1133.
- Davidovici, B., Karakis, I., Bourboulia, D., Ariad, S., Zong, J.-C., Benharrach, D., Dupin, N., Weiss, R., Sarov, B. & Boshoff, C. 2001 The seroepidemiology of KSHV among Israeli Jews. *J. Natl Cancer Inst.* **93**, 194–202.
- Davis, D. A., Humphrey, R. W., Newcomb, F. M., O'Brien, T. R., Goedert, J. J., Strauss, S. E. & Yarchoan, R. 1997a Detection of serum antibodies to a Kaposi's sarcoma-associated herpesvirus specific peptide. *J. Infect. Dis.* **175**, 1071–1079.
- Davis, M. A., Sturzl, M. A., Blasig, C., Schreier, A., Guo, H. G., Reitz, M., Opalenik, S. R. & Browning, P. J. 1997b Expression

- of human herpesvirus 8-encoded cyclin D in Kaposi's sarcoma spindle cells. *J. Natl Cancer Inst.* **89**, 1829–1831.
- De Amicis, T. 1897 Die sarkomatose der haut. *Monatsschr. prakt.* **25**, 309.
- Delabesse, E., Oksenhendler, E., Lebbé, C., Verola, O., Varet, B. & Turhan, A. G. 1997 Molecular analysis of clonality in Kaposi's sarcoma. *J. Clin. Pathol.* **50**, 664–668.
- Desrosiers, R. C., Sasseville, V. G., Czajak, S. C., Zhang, X., Mansfield, K. G., Kaur, A., Johnson, R. P., Lackner, A. A. & Jung, J. U. 1997 A herpesvirus of rhesus monkeys related to the human Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **71**, 9764–9769.
- de Thé, G. (and 10 others) 1978a Epidemiological evidence for causal relationship between Epstein–Barr virus and Burkitt's lymphoma from a Ugandan prospective study. *Nature* **274**, 756–761.
- de Thé, G., Lavoue, M. F. & Muenz, L. 1978b Differences in EBV antibody titres of patients with nasopharyngeal carcinoma originating from high, intermediate and low incidence areas. *IARC Sci. Publ.* **20**, 471–481.
- de Thé, G., Bestetti, G., Van Beveren, M. & Gessain, A. 1999 Prevalence of human herpesvirus 8 infection before the acquired immunodeficiency disease syndrome-related epidemic of Kaposi's sarcoma in east Africa. *J. Natl Cancer Inst.* **91**, 1888–1889.
- Di Alberti, L., Piattelli, A., Artese, L., Favia, G., Patel, S., Saunders, N., Porter, S. R., Scully, C. M., Ngui, S.-L. & Teo, C.-G. 1997 Human herpesvirus 8 variants in sarcoid tissues. *Lancet* **350**, 1655–1661.
- Diamond, C., Brodie, S. J., Krieger, J. N., Huang, M.-L., Koelle, D. M., Diem, K., Muthui, D. & Corey, L. 1998 Human herpesvirus 8 in the prostate glands of men with Kaposi's sarcoma. *J. Virol.* **72**, 6223–6227.
- Dictor, M., Ramech, E., Way, D., Witte, M. & Bendsoe, N. 1996 Human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) DNA in Kaposi's sarcoma lesions, AIDS Kaposi's sarcoma cell lines, endothelial Kaposi's sarcoma simulators, and the skin of immunosuppressed patients. *Am. J. Pathol.* **148**, 2009–2016.
- D'Oliveira, J. J. G. & Torres, F. O. 1972 Kaposi's sarcoma in the Bantu of Mozambique. *Cancer* **30**, 553–561.
- Du, M.-Q., Liu, H., Diss, T. C., Hamoudi, R. A., Ye, H., Dupin, N., Oksenhendler, E., Boshoff, C. & Isaacson, P. G. 2001 KSHV infects monotypic (IgM) but polyclonal naive B-cells in Castleman's disease and associated lymphoproliferative disorders. *Blood*. (In the press.)
- Dukers, N. H., Renwick, N., Prins, M., Geskus, R. B., Schulz, T. F., Weverling, G. J., Coutinho, R. A. & Goudsmit, J. 2000 Risk factors for human herpesvirus 8 seropositivity and seroconversion in a cohort of homosexual men. *Am. J. Epidemiol.* **151**, 213–224.
- Dupin, N., Grandadam, M. & Calvez, V. 1995 Herpes-like DNA sequences in patients with Mediterranean Kaposi's sarcoma. *Lancet* **345**, 761–762.
- Dupin, N. (and 12 others) 1999 Distribution of HHV-8 positive cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. *Proc. Natl Acad. Sci. USA* **96**, 4546–4551.
- Dupin, N., Diss, T., Kellam, P., Tulliez, M., Du, M.-Q., Weiss, R. A., Isaacson, P. G. & Boshoff, C. 2000 HHV-8 is associated with a plasmablastic variant of Castleman's disease that is linked to HHV-8 positive plasmablastic lymphoma. *Blood* **95**, 1406–1412.
- Ensoli, B., Salahuddin, S. Z. & Gallo, R. C. 1989 AIDS-associated Kaposi's sarcoma: a molecular model for its pathogenesis. *Cancer Cells* **1**, 93–96.
- Ensoli, B., Buonaguro, L., Barillari, G., Fiorelli, V., Gendelman, R., Morgan, R. A., Wingfield, P. & Gallo, R. C. 1993 Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J. Virol.* **67**, 277–287.
- Ensoli, B., Gendelman, R., Markham, P., Fiorelli, V., Colombini, S., Raffeld, M., Cafaro, A., Chang, H. K., Brady, J. N. & Gallo, R. C. 1994 Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. *Nature* **371**, 674–680.
- Farge, D. 1993 Kaposi's sarcoma in organ transplant recipients. The Collaborative Transplantation Research Group of Ile de France. *Eur. J. Med.* **2**, 339–343.
- Fenig, E., Brenner, B., Rakowsky, E., Lapidot, M., Katz, A. & Sulkes, A. 1998 Classic Kaposi's sarcoma: experience at Rabin Medical Center in Israel. *Am. J. Clin. Oncol.* **21**, 498–500.
- Fiorelli, V. (and 10 others) 1998 Gamma-interferon produced by CD8⁺ T cells infiltrating Kaposi's sarcoma induces spindle cells with angiogenic phenotype and synergy with human immunodeficiency virus-1 Tat protein: an immune response to human herpesvirus-8 infection? *Blood* **91**, 956–967.
- Flore, O., Rafii, S., Ely, S., O'Leary, J. J., Hyjek, E. M. & Cesarman, E. 1998 Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. *Nature* **394**, 588–592.
- Franceschi, S. & Geddes, M. 1995 Epidemiology of classic Kaposi's sarcoma, with special reference to Mediterranean population. *Tumori* **81**, 308–314.
- Franceschi, S. & Serraino, D. 1995 Kaposi's sarcoma and KSHV. *Lancet* **346**, 1360–1361.
- Frankel, A. D. & Pabo, C. O. 1988 Cellular uptake of the Tat protein from human immunodeficiency virus. *Cell* **55**, 1189–1193.
- Friborg Jr, J., Kong, W., Hottiger, M. O. & Nabel, G. J. 1999 p53 inhibition by LANA protein of KSHV protects against cell death. *Nature* **402**, 889–894.
- Frizzera, G. 1988 Castleman's disease and related disorders. *Semin. Diagn. Pathol.* **5**, 346–364.
- Gaidano, G. (and 11 others) 1996 Distribution of human herpesvirus-8 sequences throughout the spectrum of AIDS-related neoplasia. *AIDS* **10**, 941–949.
- Gaidano, G. (and 10 others) 1999 Genetic characterization of HHV-8/KSHV-positive primary effusion lymphoma reveals frequent mutations of BCL6: implications for disease pathogenesis and histogenesis. *Genes Chromosomes Cancer* **24**, 16–23.
- Gallo, R. C. 1998 The enigmas of Kaposi's sarcoma. *Science* **282**, 1837–1839.
- Ganem, D. 1996 Human herpesvirus 8 and the biology of Kaposi's sarcoma. *Semin. Virol.* **7**, 325–332.
- Gao, S. J. (and 12 others) 1996a KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. *Nature Med.* **2**, 925–928.
- Gao, S. J. (and 10 others) 1996b Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. *New Engl. J. Med.* **335**, 233–241.
- Geddes, M., Franceschi, S., Balzi, D., Arniani, S., Gafa, L. & Zanetti, R. 1995 Birthplace and classic Kaposi's sarcoma in Italy. *J. Natl. Cancer Inst.* **87**, 1015–1017.
- Gessain, A. (and 10 others) 1996 Kaposi sarcoma-associated herpes-like virus (human herpesvirus type 8) DNA sequences in multicentric Castleman's disease: is there any relevant association in non-human immunodeficiency virus-infected patients? *Blood* **87**, 414–416.
- Gessain, A. (and 14 others) 1997 Human herpes virus 8 (Kaposi's sarcoma herpes virus) and malignant lymphoproliferations in France: a molecular study of 250 cases including two AIDS-associated body cavity based lymphomas. *Leukemia* **11**, 266–272.
- Gessain, A., Mauclore, P., Van Beveren, M., Plancoulaine, S., Ayoub, A., Essame-Oyono, J. L., Martin, P. M. & de Thé,

- G. 1999 Human herpesvirus 8 primary infection occurs during childhood in Cameroon, central Africa. *Int. J. Cancer*, **81**, 189–192.
- Gill, P., Tsai, Y. C., Rao, A. P., Spruck, C. H., Zheng, T., Harrington, W. A., Cheung, T., Nathwani, B. & Jones, P. 1998 Evidence for multiclonality in multicentric Kaposi's sarcoma. *Proc. Natl Acad. Sci. USA* **95**, 8257–8261.
- Giraldo, G., Beth, E. & Hagenau, F. 1972 Herpes-type virus particles in tissue culture of Kaposi's sarcoma from different geographic regions. *J. Natl Cancer Inst.* **49**, 1509–1526.
- Giraldo, G., Kourilsky, F. M., Henle, W., Mike, V., Huraux, J. M., Andersen, H. K., Gharbi, M. R., Kyalwazi, S. K. & Puissant, A. 1975 Antibody patterns to herpesviruses in Kaposi's sarcoma: serological association of European Kaposi's sarcoma with cytomegalovirus. *Int. J. Cancer* **15**, 839–848.
- Greensill, J., Sheldon, J. A., Renwick, N. M., Beer, B. E., Norley, S., Goudsmit, J. & Schulz, T. F. 2000 Two distinct gamma-2 herpesviruses in African green monkeys; a second gamma-2 herpesvirus lineage among Old World primates? *J. Virol.* **74**, 1572–1577.
- Grulich, A. E., Beral, V. & Sverdlow, A. J. 1992 Kaposi's sarcoma in England and Wales before the AIDS epidemic. *Br. J. Cancer* **66**, 1135–1137.
- Harwood, A. R., Osoba, D., Hofstadter, S. L., Goldstein, M. B., Cardella, C. J., Holecek, M. J., Kunynetz, R. & Gianinice, R. A. 1979 Kaposi's sarcoma in recipients of renal transplants. *Am. J. Med.* **67**, 759–765.
- Hayward, G. S. 1999 KSHV strains: the origins and global spread of the virus. In *Seminars in cancer biology: Kaposi's sarcoma-associated herpesvirus*, vol. 9 (ed. R. A. Weiss & C. Boshoff), pp. 187–199. London: Academic Press.
- He, J., Bhat, G., Kankasa, C., Chintu, C., Mitchell, C., Duan, W. & Wood, C. 1998 Seroprevalence of human herpesvirus 8 among Zambian women of childbearing age without Kaposi's sarcoma (KS) and mother-child pairs with KS. *J. Infect. Dis.* **178**, 1787–1790.
- Howard, M. R. (and 11 others) 1997 Detection of human herpesvirus 8 DNA in semen from HIV-infected individuals but not healthy semen donors. *AIDS* **11**, F15–F19.
- Huang, Y. Q., Li, J. J., Rush, M. G., Poiesz, B. J., Nicolaides, A., Jacobson, M., Zhang, W. G., Coutavas, E., Abbott, M. A. & Friedman, Kien, A. E. 1992 HPV-16-related DNA sequences in Kaposi's sarcoma. *Lancet* **339**, 515–518.
- IARC 1997 *Monographs on the evaluation of carcinogenic risks to humans, Epstein-Barr virus and Kaposi's sarcoma herpesvirus/human herpesvirus*, no. 8. Lyon, France: IARC Press.
- Iscovich, J., Bofetta, P., Winkelmann, R., Brennan, P. & Azizi, E. 1998 Classic Kaposi's sarcoma in Jews living in Israel, 1961–1989: a population-based incidence study. *AIDS* **12**, 2067–2072.
- Jaffe, E. S. 1996 Primary body cavity-based AIDS-related lymphomas. *Am. J. Pathol.* **105**, 141–143.
- Jenner, R., Alba, M. M., Boshoff, C. & Kellam, P. 2001 Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. *J. Virol.* **75**, 891–902.
- Jin, Y. T., Tsai, S. T., Yan, J. J., Hsiao, J. H., Lee, Y. Y. & Su, I. J. 1996 Detection of Kaposi's sarcoma-associated herpesvirus-like DNA sequence in vascular lesions. A reliable diagnostic marker for Kaposi's sarcoma. *Am. J. Clin. Pathol.* **105**, 360–363.
- Jones, D., Ballestas, M. E., Kaye, K. M., Gulizia, J. M., Winters, G. L., Fletcher, J., Scadden, D. T. & Aster, J. C. 1998 Primary effusion lymphoma and Kaposi's sarcoma in a cardiac-transplant recipient. *New Engl. J. Med.* **339**, 444–449.
- Judde, J.-G., Lacoste, V., Briere, J., Kassa-Kelembho, E., Clyti, E., Couppie, P., Buchrieser, C., Tuilliez, M., Morvan, J. & Gessain, A. 2000 Monoclonality or oligoclonality of human herpesvirus 8 terminal repeat sequences in Kaposi's sarcoma and other diseases. *J. Natl Cancer Inst.* **9**, 729–736.
- Jussila, L. (and 11 others) 1998 Lymphatic endothelium and Kaposi's sarcoma spindle cells detected by antibodies against the vascular endothelial growth factor receptor-3. *Cancer Res.* **58**, 1599–1604.
- Kaposi, M. 1872 Idiopathisches multiples pigmentsarcom der haut. *Arch. Dermatol. Syphilis* **4**, 265–273.
- Karcher, D. S., Dawkins, F. & Garrett, C. T. 1992 Body cavity-based non-Hodgkin's lymphoma (NHL) in HIV-infected patients: B-cell lymphoma with unusual clinical, immunophenotypic, and genotypic features. *Lab. Invest.* **92**, 80a.
- Katano, H., Sato, Y., Kurata, T., Mori, S. & Sata, T. 2000 Expression and localization of human herpesvirus-8-encoded proteins in primary effusion lymphoma, Kaposi's sarcoma, and multicentric Castleman's disease. *Virology* **269**, 335–344.
- Kedes, D. H., Operskalski, E., Busch, M., Kohn, R., Flood, J. & Ganem, D. 1996 The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. *Nat. Med.* **2**, 918–924.
- Kedes, D. H., Ganem, D., Ameli, N., Bacchetti, P. & Greenblatt, R. 1997a The prevalence of serum antibody to human herpesvirus 8 (Kaposi sarcoma-associated herpesvirus) among HIV-seropositive and high-risk HIV-seronegative women. *J. Am. Med. Assoc.* **277**, 478–481.
- Kedes, D. H., Lagunoff, M., Renne, R. & Ganem, D. 1997b Identification of the gene encoding the major latency-associated nuclear antigen of the Kaposi's sarcoma-associated herpesvirus. *J. Clin. Invest.* **100**, 2606–2610.
- Kellam, P., Boshoff, C., Whitby, D., Matthews, S., Weiss, R. A. & Talbot, S. J. 1997 Identification of a major latent nuclear antigen (LNA-1) in the human herpesvirus 8 (HHV-8) genome. *J. Hum. Virol.* **1**, 19–29.
- Kellam, P., Bourboulia, D., Dupin, N., Talbot, S., Boshoff, C. & Weiss, R. A. 1999 Characterising monoclonal antibodies against KSHV latent nuclear antigen (LNA-1). *J. Virol.* **73**, 5149–5155.
- Kempf, W., Adams, V., Pfaltz, M., Briner, J., Schmid, M., Moos, R. & Hassam, S. 1995 Human herpesvirus type 6 and cytomegalovirus in AIDS-associated Kaposi's sarcoma: no evidence for an etiological association. *Hum. Pathol.* **26**, 914–919.
- Knowles, D. M., Inghirami, G., Ubriaco, A. & Dalla-Favera, R. 1989 Molecular genetic analysis of three AIDS-associated neoplasms of uncertain lineage demonstrates their B-cell derivation and the possible pathogenetic role of the Epstein-Barr virus. *Blood* **73**, 792–799.
- Koelle, D. M., Huang, M.-L., Chandran, B., Vieira, J., Piepkorn, M. & Corey, L. 1997 Frequent detection of Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) in saliva of human immunodeficiency virus-infected men: clinical and immunologic correlates. *J. Infect. Dis.* **176**, 94–102.
- Komanduri, K. V., Luce, J. A., McGrath, M. S., Herndier, B. G. & Ng, V. L. 1996 The natural history and molecular heterogeneity of HIV-associated primary malignant lymphomatous effusions. *J. AIDS Hum. Retrovirol.* **13**, 215–226.
- Koulbaly, M., Kabba, I. S., Cisse, A., Diallo, S. B., Diallo, M. B., Keita, N., Camara, N. D., Diallo, M. S., Sylla, B. S. & Parkin, D. M. 1997 Cancer incidence in Conakry, Guinea: first results from the Cancer Registry 1992–1995. *Int. J. Cancer* **70**, 39–45.
- Lacoste, V., Kadyrova, E., Chistiakova, I., Gurtsevitch, V., Judde, J.-G. & Gessain, A. 2000a Molecular characterisation of Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8 strains from Russia. *J. Gen. Virol.* **81**, 1217–1222.
- Lacoste, V., Maucel, P., Dubreuil, G., Lewis, J., Courbot-Georges, M.-C. & Gessain, A. 2000b 88 KSHV-like herpesviruses in chimps and gorillas. *Nature* **407**, 151–152.

- Lebbé, C., deCremoux, P., Rybojad, M., Costa da Cunha, C., Morel, P. & Calvo, F. 1995 Kaposi's sarcoma and new herpesvirus. *Lancet* **345**, P1180.
- Lebbé, C., Agbalika, F., de Cremoux, P., Deplanche, M., Rybojad, M., Masgrau, E., Morel, P. & Calvo, F. 1997 Detection of human herpesvirus 8 and human T-cell lymphotropic virus type 1 sequences in Kaposi sarcoma. *Arch. Dermatol.* **133**, 25–30.
- Lennette, E. T., Blackbourn, D. J. & Levy, J. A. 1996 Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. *Lancet* **348**, 858–861.
- Li, J. J., Huang, Y. Q., Cockerell, C. J. & Friedman Kien, A. E. 1996 Localization of human herpes-like virus type 8 in vascular endothelial cells and perivascular spindle-shaped cells of Kaposi's sarcoma lesions by *in situ* hybridization. *Am. J. Pathol.* **148**, 1741–1748.
- Lin, B. T. Y., Chen, Y. Y., Battifora, H. & Weiss, L. M. 1996 Absence of Kaposi's sarcoma-associated herpesvirus-like DNA sequences in malignant vascular tumors of the serous membranes. *Mod. Pathol.* **9**, 1143–1146.
- Lin, J.-C., Lin, S.-C., Mar, E.-C., Pellett, P. E., Stamey, F. R., Stewart, J. A. & Spira, T. J. 1998 Retraction: is KSHV in semen of HIV-infected homosexual men? *Lancet* **351**, 1365.
- Luppi, M., Barozzi, P., Maiorana, A., Collina, G., Ferrari, M. G., Marasca, R., Morselli, M., Rossi, E., Ceccherini Nelli, L. & Torelli, G. 1996 Frequency and distribution of herpesvirus-like DNA sequences (KSHV) in different stages of classic Kaposi's sarcoma and in normal tissues from an Italian population. *Int. J. Cancer* **66**, 427–431.
- McDonagh, D. P., Liu, J., Gaffey, M. J., Layfield, L. J., Azumi, N. & Traweck, S. T. 1996 Detection of Kaposi's sarcoma-associated herpesvirus-like DNA sequence in angiosarcoma. *Am. J. Pathol.* **149**, 1363–1368.
- McGeoch, D. J. & Davidson, A. J. 1999 The descent of human herpesvirus 8. In *Seminars in cancer biology*, vol. 9 (ed. R. A. Weiss & C. Boshoff), pp. 201–209. London: Academic Press.
- Marchio, S., Primo, L., Pagano, M., Palestro, G., Albini, A., Veikkola, T., Cascone, I., Alitalo, K. & Bussolino, F. 1999 Vascular endothelial growth factor-C stimulates the migration and proliferation of Kaposi's sarcoma cells. *J. Biol. Chem.* **274**, 27 617–27 622.
- Marill, F. G., Sayag, J. & Hadida, E. 1973 L'acro-sarcomatose de Kaposi en Algérie. *Med. Trop.* **33**, 471–481.
- Martin, J. N., Ganem, D. E., Osmond, D. H., Page-Shafer, K. A., Macrae, D. & Kedes, D. H. 1998 Sexual transmission and the natural history of human herpesvirus 8 infection. *New Engl. J. Med.* **338**, 948–954.
- Mayama, S., Cuevas, L. E., Sheldon, J., Omar, O. H., Smith, D. H., Okong, P., Silvel, B., Hart, C. A. & Schulz, T. F. 1998 Prevalence and transmission of Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) in Ugandan children and adolescents. *Int. J. Cancer* **11**, 817–820.
- Meng, Y.-X. (and 11 others) 1999 Individuals from North America, Australasia, and Africa are infected with four different genotypes of human herpesvirus-8. *Virology* **261**, 106–119.
- Miles, S. A. (and 10 others) 1990 AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. *Proc. Natl Acad. Sci. USA* **87**, 4068–4072.
- Monini, P., de Lellis, L., Fabris, M., Rigolin, F. & Cassai, E. 1996a Kaposi's sarcoma-associated herpesvirus DNA sequences in prostate tissue and human semen. *New Engl. J. Med.* **334**, 1168–1172.
- Monini, P., Rotola, A., de Lellis, L., Corallini, A., Secchiero, P., Albini, A., Benelli, R., Parravicini, C., Barbanti-Brodano, G. & Cassai, E. 1996b Latent BK virus infection and Kaposi's sarcoma pathogenesis. *Int. J. Cancer* **66**, 717–722.
- Moore, P. S. & Chang, Y. 1995 Detection of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma lesions from persons with and without HIV infection. *New Engl. J. Med.* **332**, 1181–1185.
- Moore, P. S., Kingsley, L. A., Holmberg, S. D., Spira, T., Gupta, P., Hoover, D. R., Parry, J. P., Conley, L. J., Jaffe, H. W. & Chang, Y. 1996 Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma. *AIDS* **10**, 175–180.
- Moses, A. V., Fish, K. N., Ruhl, R., Smith, P. P., Strussenberg, J. G., Zhu, L., Chandran, B. & Nelson, J. A. 1999 Long-term infection and transformation of dermal microvascular endothelial cells by human herpesvirus 8. *J. Virol.* **73**, 6892–6902.
- Nador, R. G., Cesarman, E., Chadburn, A., Dawson, D. B., Ansari, M. Q., Said, J. & Knowles, D. M. 1996 Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. *Blood* **88**, 645–656.
- Nair, B. C., DeVico, A. L., Nakamura, S., Copeland, T. D., Chen, Y., Patel, A., O'Neil, T., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. 1992 Identification of a major growth factor for AIDS-Kaposi's sarcoma cells as oncostatin M. *Science* **255**, 1430–1432.
- Newton, R., Ngilimana, P. J., Grulich, A., Beral, V., Sindikubwabo, B., Nganyira, A. & Parkin, D. M. 1996 Cancer in Rwanda. *Int. J. Cancer* **66**, 75–81.
- Nicholas, J. (and 11 others) 1998 Novel organizational features, captured cellular genes, and strain variability within the genome of KSHV/HHV-8. *J. Natl Cancer Inst.* **23**, 79–88.
- Nickloff, B. J. & Griffiths, C. E. 1989 The spindle-shaped cells in cutaneous Kaposi's sarcoma. Histologic simulators include factor XIIIa dermal dendrocytes. *Am. J. Pathol.* **135**, 793–800.
- O'Brien, T. R., Kedes, D., Ganem, D., Macrae, D. R., Rosenberg, P. S., Molden, J. & Goedert, J. J. 1999 Evidence for concurrent epidemics of human herpesvirus 8 and human immunodeficiency virus type 8 in US homosexual men: rates, risk factors, and relationship to Kaposi's sarcoma. *J. Infect. Dis.* **180**, 1010–1017.
- Oettle, A. G. 1962 Geographic and racial differences in the frequency of Kaposi's sarcoma as evidence of environmental or genetic causes. In *Symposium on Kaposi's sarcoma* (ed. L. V. Ackerman & J. F. Murray). Basel, Switzerland: Karger.
- Oksenhendler, E., Cazals-Hatem, D., Schultz, T. F., Barateau, V., Grollet, L., Sheldon, J., Clauvel, J.-P., Sigaux, F. & Agbalika, F. 1998 Transient angiolymphoid hyperplasia and Kaposi's sarcoma after primary infection with human herpesvirus 8 in a patient with human immunodeficiency virus infection. *New Engl. J. Med.* **338**, 1585–1591.
- Olsen, S., Chang, Y., Moore, P., Biggar, R. & Melbye, M. 1998 Increasing Kaposi's sarcoma-associated herpesvirus seroprevalence with age in a highly Kaposi's sarcoma endemic region, Zambia in 1985. *AIDS* **12**, 1921–1925.
- Olweny, C. L. M., Kaddumukasa, A., Atine, O., Owor, R., Magrath, I. T. & Ziegler, J. 1976 Childhood Kaposi's sarcoma in Uganda: clinical features and treatment. *Br. J. Cancer* **33**, 555–560.
- O'Neil, E., Henson, T. H., Ghorbani, A. J., Land, M. A., Webber, B. L. & Garcia, J. V. 1996 Herpes virus-like sequences are specifically found in Kaposi sarcoma lesions. *J. Clin. Pathol.* **49**, 306–308.
- Parkin, D. M., Wabinga, H., Namboozee, S. & Wabwire-Mangen, F. 1999 AIDS-related cancers in Africa: maturation of the epidemic in Uganda. *AIDS* **13**, 2563–2570.
- Parravicini, C., Lauri, E., Baldini, L., Neri, A., Poli, F., Sirchia, G., Moroni, M., Galli, M. & Corbellino, M. 1997 Kaposi's sarcoma-associated herpesvirus infection and multiple myeloma. *Science* **278**, 1969.

- Parravicini, C., Corbellino, M., Paulli, M., Magrini, U., Lazzarino, M., Moore, P. S. & Chang, Y. 1998 Expression of a virus-derived cytokine, KSHV vIL-6, in HIV seronegative Castleman's disease. *Am. J. Pathol.* **6**, 1517–1522.
- Parravicini, C., Chandran, B., Corbellino, M., Berti, E., Paulli, M., Moore, P. S. & Chang, Y. 2000 Differential viral protein expression in Kaposi's sarcoma-associated herpesvirus-infected diseases: Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease. *Am. J. Pathol.* **156**, 743–749.
- Pellet, P. E. (and 13 others) 1999 Multicenter comparison of PCR assays for detection of human herpesvirus 8 DNA in semen. *J. Clin. Microbiol.* **37**, 1298–1301.
- Penn, I. 1983 Kaposi's sarcoma in immuno-suppressed patients. *J. Clin. Lab. Immunol.* **12**, 1–10.
- Petersen, G. M., Rotter, J. I., Cantor, R. M., Field, L. L., Greenwald, S., Lim, J. S., Roy, C., Schoenfeld, V., Lowden, J. A. & Kaback, M. M. 1983 The Tay–Sachs disease gene in North American Jewish populations: geographic variations and origin. *Am. J. Hum. Genet.* **35**, 1258–1269.
- Plancoulaine, S., Abel, L., Van Beveren, M., Tregouet, A., Joubert, M., Tortevoe, P., de Thé, G. & Gessain, A. 2000 Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population. *Lancet* **356**, 1062–1065.
- Platt, G. M., Simpson, G. R., Mitnacht, S. & Schulz, T. F. 1999 Latent nuclear antigen of Kaposi's sarcoma-associated herpesvirus interacts with RING3, a homolog of the *Drosophila* female sterile homeotic (fsh) gene. *J. Virol.* **73**, 9789–9795.
- Poole, L. J., Zong, J.-C., Ciufu, D. M., Alcendor, D. J., Cannon, J. S., Ambinder, R., Orenstein, J. M., Reitz, M. S. & Hayward, G. S. 1999 Comparison of genetic variability at multiple loci across the genomes of the major subtypes of Kaposi's sarcoma-associated herpesvirus reveals evidence for recombination and for two distinct types of open reading frame K15 alleles at the right-hand end. *J. Virol.* **73**, 6646–6660.
- Prakash, O., Tang, Z.-Y., He, Y. E., Ali, M. S., Coleman, R., Gill, J., Farr, G. & Samaniego, F. 2000 Human Kaposi's sarcoma cell-mediated tumorigenesis in human immunodeficiency type 1 Tat-expressing transgenic mice. *J. Natl Cancer Inst.* **9**, 721–728.
- Qunibi, W., Akhtar, M., Sheth, K., Ginn, H. E., Al-Furayh, O., DeVol, E. B. & Taher, S. 1988 Kaposi's sarcoma: the most common tumor after renal transplantation in Saudi Arabia. *Am. J. Med.* **84**, 225–232.
- Raab, M.-S., Albrecht, J.-C., Birkmann, A., Yaguboglu, S., Lang, D., Fleckenstein, B. & Neipel, F. 1998 The immunogenic glycoprotein gp35-37 of human herpesvirus 8 is encoded by open reading frame K8. Part I. *J. Virol.* **72**, 6725–6731.
- Raab-Traub, N. & Flynn, K. 1986 The structure of the termini of the Epstein–Barr virus as a marker of clonal cellular proliferation. *Cell* **47**, 883–889.
- Rabkin, C. S., Bedi, G., Musaba, E. & Biggar, R. J. 1995 AIDS-related Kaposi's sarcoma is a clonal neoplasm. *New Engl. J. Med.* **1**, 257–260.
- Rabkin, C. S., Janz, S., Lash, A., Coleman, A. E., Musaba, E., Liotta, L., Biggar, R. J. & Zhuang, Z. 1997 Monoclonal origin of multicentric Kaposi's sarcoma lesions. *New Engl. J. Med.* **336**, 988–993.
- Radkov, S., Kellam, P. & Boshoff, C. 2000 The latent nuclear antigen of KSHV targets the retinoblastoma/E2F pathway and with H-Ras transforms primary rat cells. *Nature Med.* **6**, 1121–1127.
- Rainbow, L., Platt, G. M., Simpson, G. R., Sarid, R., Gao, S.-J., Stoiber, H., Herrington, C. S., Moore, P. S. & Schulz, T. F. 1997 The 222–234 kd nuclear protein (LNA) of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) is encoded by orf 73 and a component of the latency-associated nuclear antigen. *J. Virol.* **71**, 5915–5921.
- Renne, R., Zhong, W., Herndier, B., McGrath, M., Abbey, N., Kedes, D. & Ganem, D. 1996 Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. *Nat. Med.* **2**, 342–346.
- Renwick, N., Halaby, T., Weverling, G. J., Dukers, N. H., Simpson, G. R., Coutinho, R. A., Lange, J. M., Schulz, T. F. & Goudsmit, J. 1998 Seroconversion to human herpesvirus-8 during HIV infection is highly predictive of Kaposi's sarcoma. *AIDS* **12**, 2481–2488.
- Rettig, M. B. (and 11 others) 1997 Kaposi's sarcoma associated herpesvirus infection of bone marrow dendritic cells from multiple myeloma. *Science* **276**, 1851–1854.
- Rezza, G. (and 10 others) 2000 Prevalence and risk factors for human herpesvirus 8 infection in northern Cameroon. *Sex Transm. Dis.* **27**, 159–164.
- Risau, W. 1997 Mechanisms of angiogenesis. *Nature* **386**, 671–674.
- Rose, T. M., Strand, K. B., Schultz, E. R., Schaefer, G., Rankin Jr, G. W., Thouless, M. E., Tsai, C. C. & Bosch, M. L. 1997 Identification of two homologs of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in retroperitoneal fibromatosis of different macaque species. *J. Virol.* **71**, 4138–4144.
- Rothman, S. 1962 Some clinical aspects of Kaposi's sarcoma in the European and North-American population. In *Symposium on Kaposi's sarcoma* (ed. L. V. Ackerman & J. F. Murray). Basel, Switzerland: Karger.
- Russo, J. J. (and 10 others) 1996 Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc. Natl Acad. Sci. USA* **93**, 14 862–14 867.
- Said, J. W., Tasuka, T., Takeachi, S., Asou, H., de Vos, S., Cesarman, E., Knowles, D. M. & Koeffler, H. P. 1996 Primary effusion lymphoma in women: report of two cases of Kaposi's sarcoma herpesvirus-associated effusion-based lymphoma in human immunodeficiency virus-negative women. *Blood* **88**, 3124–3128.
- Salahuddin, S. Z., Nakamura, S., Biberfeld, P., Kaplan, M. H., Markham, P. D., Larsson, L. & Gallo, R. C. 1988 Angiogenic properties of Kaposi's sarcoma-derived cells after long-term culture *in vitro*. *Science* **242**, 430–433.
- Samaniego, F., Markham, P. D., Gallo, R. C. & Ensoli, B. 1995 Inflammatory cytokines induce AIDS–Kaposi's sarcoma-derived spindle cells to produce and release basic fibroblast growth factor and enhance Kaposi's sarcoma-like lesion formation in nude mice. *J. Immunol.* **154**, 3582–3592.
- Schalling, M., Ekman, M., Kaaya, E. E., Linde, A. & Biberfeld, P. 1995 A role for a new herpes virus (KSHV) in different forms of Kaposi's sarcoma. *Nature Med.* **1**, 705–706.
- Simpson, G. R. (and 16 others) 1996 Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. *Lancet* **348**, 1133–1138.
- Sirianni, M. C., Uccini, S., Angeloni, A., Faggioni, A., Cottoni, F. & Ensoli, B. 1997 Circulating spindle cells: correlation with human herpesvirus-8 (HHV-8) infection and Kaposi's sarcoma. *Lancet* **349**, 255.
- Sitas, F. (and 11 others) 1999 Antibodies against human herpesvirus-8 in black South African patients with cancer. *New Engl. J. Med.* **340**, 1863–1871.
- Smith, N., Sabin, C. A., Bourboulia, D., Boshoff, C., Peters, B., deRuiter, A., Weiss, R. A., Best, J. A. & Whitby, D. 1999 Lack of evidence for transmission of HHV-8 by heterosexual sex. *J. Infect. Dis.* **180**, 600–606.
- Soulhier, J., Grollet, L., Oksenhendler, E., Cacoub, P., Cazals Hatem, D., Babinet, P., d'Agay, M. F., Clauvel, J. P., Raphael,

- M. & Degos, L. 1995 Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. *Blood* **86**, 1276–1280.
- Staskus, K. A. (and 10 others) 1997 Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. *J. Virol.* **71**, 715–719.
- Strauchen, J. A., Hauser, A. D., Burstein, D., Jimenez, R., Moore, P. S. & Chang, Y. 1997 Body cavity-based malignant lymphoma containing Kaposi sarcoma-associated herpesvirus in an HIV-negative man with previous Kaposi's sarcoma. *Annls Int. Med.* **125**, 822–825.
- Sturzl, M., Brandstetter, H. & Roth, W. K. 1992 Kaposi's sarcoma: a review of gene expression and ultrastructure of KS spindle cells *in vivo*. *AIDS Res. Hum. Retroviruses* **8**, 1753–1764.
- Sturzl, M. (and 12 others) 1997 Expression of HHV-8 latency-associated T0.7 RNA in spindle cells and endothelial cells of AIDS-associated, classical and African Kaposi's sarcoma (KS). *Int. J. Cancer* **72**, 68–71.
- Sturzl, M., Hohendi, C., Zietz, C., Castanos-Velez, E., Wunderlich, A., Ascherl, G., Biberfeld, P., Monini, P., Browning, P. J. & Ensoli, B. 1999 Expression of K13/v-FLIP gene of human herpesvirus 8 and apoptosis in Kaposi's sarcoma spindle cells. *J. Natl Cancer Inst.* **20**, 1725–1733.
- Tarte, K., Olsen, S. J., Rossi, J. F., Legouffe, E., Lu, Z. Y., Jourdan, M., Chang, Y. & Klein, B. 1998 Kaposi's sarcoma-associated herpesvirus is not detected with immunosuppression in multiple myeloma. *Blood* **92**, 2186–2188.
- Tasaka, T., Said, J. W. & Koeffler, H. P. 1996 Absence of HHV-8 in prostate and semen. *New Engl. J. Med.* **335**, 1237–1238.
- Tonin, P. (and 24 others) 1996 Frequency of recurrent BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer families. *Nat. Med.* **2**, 1179–1183.
- US Public Health Service 1981 Kaposi's sarcoma and *Pneumocystis pneumonia* among homosexual men in New York City and California. *Morbid. Mortal. Weekly Rep.* **30**, 305–308.
- Uthman, A., Brna, C., Weninger, W. & Tschachler, E. 1996 No HHV8 in non-Kaposi's sarcoma mucocutaneous lesions from immunodeficient HIV-positive patients. *Lancet* **347**, 1700–1701.
- Vaishnav, Y. N. & Wong-Staal, F. 1991 The biochemistry of AIDS. *A. Rev. Biochem.* **60**, 577–630.
- Viviano, E., Vitale, F., Ajello, F., Perna, A. M., Villafrate, M. R., Bonura, F., Arico, M., Mazzola, G. & Romano, N. 1997 Human herpesvirus type 8 DNA sequences in biological samples of HIV-positive and negative individuals in Sicily. *AIDS* **11**, 607–612.
- Vogel, J., Hinrichs, S. H., Reynolds, R. K., Luciw, P. A. & Jay, G. 1988 The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature* **335**, 606–611.
- Vogelstein, B., Fearon, E. R., Hamilton, S. R. & Feinberg, A. P. 1985 Use of restriction fragment length polymorphisms to determine the clonal origin of human tumors. *Science* **227**, 642–645.
- Wabinga, H. R., Parkin, D. M., Wabwire-Mangen, F. & Mugerwa, J. W. 1993 Cancer in Kampala, Uganda in 1989–91: changes in incidence in the era of AIDS. *Int. J. Cancer* **54**, 23–36.
- Walts, A. E., Shintaku, P. & Said, J. W. 1990 Diagnosis of malignant lymphoma in effusions from patients with AIDS by gene rearrangement. *Am. J. Clin. Pathol.* **194**, 170–175.
- Weninger, W. (and 10 others) 1999 Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells. *Lab. Invest.* **79**, 243–251.
- Whitby, D. (and 14 others) 1995 Detection of Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *Lancet* **346**, 799–802.
- Whitby, D., Boshoff, C., Luppi, M. & Torelli, G. 1997 Kaposi's sarcoma-associated herpesvirus infection and multiple myeloma. *Science* **278**, 1971–1972.
- Whitby, D., Luppi, M., Barozzi, P., Boshoff, C., Weiss, R. A. & Torelli, G. 1998 HHV-8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. *J. Natl. Cancer Inst.* **90**, 395–397.
- Whitby, D., Luppi, M., Sabin, C., Barozzi, P., Di Biase, A. R., Balli, F., Cucci, F., Weiss, R. A., Boshoff, C. & Torelli, G. 2000 Detection of antibodies to human herpesvirus 8 in Italian children: evidence for horizontal transmission. *Br. J. Cancer* **82**, 702–704.
- Zahger, D., Lotan, C., Admon, D., Klapholz, L., Kaufman, B., Shimon, D., Woolfson, N. & Gotsman, M. S. 1993 Very early appearance of Kaposi's sarcoma after cardiac transplantation in Sephardic Jews. *Am. Heart J.* **126**, 999–1000.
- Zhu, L., Wang, R., Sweat, A., Goldstein, E., Horvat, R. & Chandran, B. 1999 Comparison of human sera reactivities in immunoblots with recombinant human herpesvirus (HHV)-8 proteins associated with the latent (ORF73) and lytic (ORFs 65, K8.1A, K8.1B) replicative cycles and in immunofluorescence assays with HHV-8-infected BCBL-1 cells. *Virology* **256**, 381–392.
- Ziegler, J. L. & Katongole-Mbidde, E. 1996 Kaposi's sarcoma in childhood: an analysis of 100 cases from Uganda and relationship to HIV infection. *Int. J. Cancer* **65**, 200–203.
- Ziegler, J. L. (and 12 others) 1997 Risk factors for Kaposi's sarcoma in HIV positive subjects in Uganda. *AIDS* **11**, 1619–1626.
- Zietz, C., Bogner, J. R., Goebel, F.-D. & Lohrs, U. 1999 An unusual cluster of cases of Castleman's disease during highly active antiretroviral therapy for AIDS. *New Engl. J. Med.* **340**, 1923–1924.
- Zong, J.-C. (and 16 others) 1999 High level variability in the ORF-K1 membrane protein gene at the left end of the Kaposi's sarcoma-associated herpesvirus (HHV-8) genome defines four major virus subtypes and multiple clades in different human populations. *J. Virol.* **73**, 4156–4170.